Integration of advanced off-line and on-line systems for the monitoring of surface and drinking water quality

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Spectroscopes send out a variety of wavelengths, like scouts into a foreign land.
Inevitably, a few of these scouts do not come back
Esther Inglis-Arkell

Abstract

The main objective of this thesis is the development of new methodologies and the testing of advanced technology so final users can use this information when establishing their optimum water quality monitoring strategy. The research project was located in Barcelona (Spain) and the Llobregat River, one of the main sources for drinking water but characterised by low quality values due to its low flows and high anthropogenic impact.

Automatic on-line instruments were tested, such as the measurement of global toxicity or the recording of the spectrophotometry fingerprint, for their use as Early Warning Systems. For the identification of emerging substances, methodologies based on advanced analytical instruments at the laboratory were proposed. New indexes to describe the quality of freshwater in terms of chemical risk for Ecosystems and Human Health are provided. These indexes have been established in order to assess and define the efficiency of water treatment technologies.

Keywords

Water quality monitoring, toxicity, spectrophotometry, pharmaceuticals, risk assessment, Llobregat River, Barcelona, surface water, drinking water

Preface

Legislation, social and environmental awareness, and optimisation of treatment technologies are the main driving forces that are prompting the creation of monitoring strategies for measuring water quality.

The optimum strategy will be a combination of different technologies depending on the needs and characteristics of the system to be monitored. Constraints like cost reduction; the need to obtain results in real time; and the acquisition of accurate and sensitive data, will influence the final decision. Thanks to new technological advances, the traditional strategy based on grab sampling and analysis in the laboratory,communicating results remotely on a real-time basis, will be replaced by on-line instruments. However, currently this automation of monitoring activities is only possible for certain water quality paremetres.

A unique optimum strategy does not exist. The final solution will be based on a combination of on-line and off-line techniques for the monitoring of water quality depending on the specificity and the needs to be met for each case. Classical monitoring is based on reporting data on the concentration values of a list of substances, but this may not be enough to ascertain a general view on the quality status of a water stream. The measurement of global paremetres that provide indirect information about the general quality status or the presence of toxic substances, and other complementary approaches based on the creation of indexes to perform risk assessment, will generate information that can be more useful for final users.

This work dealt with the optimisation and validation of some of these techniques, from the optimisation of laboratory methodologies for reporting values on emerging substances to the validation of automated instruments to monitor global toxicity or to track changes in the content of organic matter. The development of indexes to assess human health and environmental chemical risk is also included. The research was focused on the Llobregat River to monitor natural waters or drinking waters after treatment steps.

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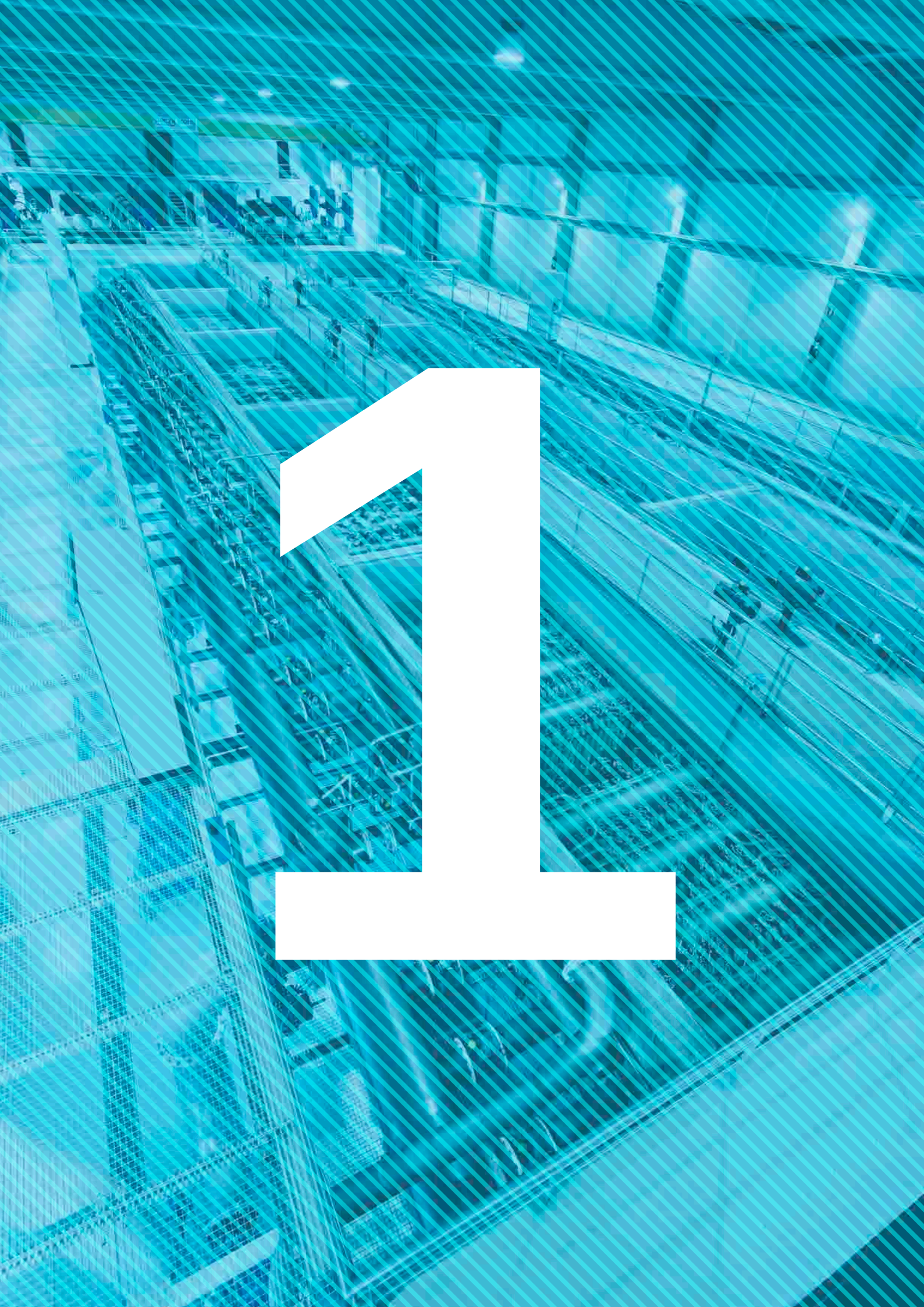
List of acronyms

AA	Annual Average
ACA	Agència Catalana de l'Aigua
AF	Assessment Factor
AOC	Assimilable Organic Carbon
AP	Alkylphenol
APEO	Alkylphenol Ethoxylates
ATP	Adenosine Triphosphate
ATS	Amphetamine Type Stimulants
BE	Benzoylecgonine
BCF	Bioconcentration Factor
BDO	Biological Oxygen Demand
BLM	Biotic Ligand Model
BMA	Barcelona Metropolitan Area
BOD	Biological Oxygen Demand
BPA	Bisphenol A
CBRN	Chemical, Biological, Radiological and Nuclear
CCD	Charge Coupled Device
CDEA	Coconut Diethanol Amides
CFIS	Continuous Flow Integrative Sampler
CFU	Colony Forming Units
CID	Collision Induced Dissociation
COD	Carbon Oxygen Demand
DBP	Disinfection By-Product
DEP	Diethylphthalate
DGT	Diffusive Gradient Thin Films
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
DWTP	Drinking Water Treatment Plant
EC50	Effective Concentration 50%
ECL	Electrochemiluminescence
EDC	Endocrine Disrupting Compounds
EDR	Electrodialysis Reversal
ELFA	Enzyme-Linked Fluorescent Immunoassays
ELISA	Enzyme-Linked Immunosorbent Assay
EQS	Environmental Quality Standards
ERA	Ecological Risk Assessment

EROD	Ethoxyresorufin O-Deethylase Activity
ESI	Electrospray Ionisation
ESRIF	European of Security Research and Innovation Forum
EWS	Early Warning Systems
FISH	Fluorescence In-situ Hybridisation
GAC	Granulated Active Carbon
GC	Gas Chromatography
GIS	Geographical Information Systems
GLM	Generalised Linear Models
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotient
ICP	Inductively Coupled Plasma
ICT	Information and Communication Technologies
IP	Identification Point
IRA	Integrated Risk Assessment
ISO	International Standard Organisation
JRC	Joint Research Centre
LAS	Linear Alkylbencene Sulphonates
LC	Liquid Chromatography
LC50	Lethal Concentration 50%
LCA	Life Cycle Analysis
LCC	Life Cycle Cost
LIT	Linear Ion Trap
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOD	Limit of Detection
LOQ	Limit of Quantification
LSD	Lysergic Acid
LTRS	Laser Tweezer Raman Spectroscopy
MAC	Maximum Allowable Concentration
MALS	Multi-Angle Light Scattering
M.E.R.	Méthode Enzymatique Rapide
MF	Microfiltration
MIP	Molecularly Imprinted Polymers
MPN	Most Probable Number
mRNA	Messenger RiboNucleic Acid
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NASBA	Nucleic Acid Sequence Based Amplification
NF	Nanofiltration

NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOM	Natural Organic Matter
NP	Nonylphenol
NP2EC	Nonylphenoxy Dicarboxylate
NPEC	Nonylphenol Carboxylate
NPEO	Nonylphenol Ethoxylates
NSAID	Non-steroidal anti-inflammatories
NTU	Nephelometric Turbidity Units
PCA	Principal Component Analysis
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PCP	Phencyclidine
PCR	Polymerase Chain Reaction
PEC	Predicted Environmental Concentration
PLS	Partial-Least-Square regression
PNEC	Predicted Non Effect Concentration
POCIS	Polar Organic Chemical Integrative Sampler
PPCP	Pharmaceutical and Personal Care Products
RAIS	Risk Assessment Information System
REACH	Registration, Evaluation and Authorisation of Chemicals
REM	Remineralisation
RfD	Reference Dose
RO	Reverse Osmosis
rRNA	Ribosomal RiboNucleic Acid
RYA	Recombinant Yeast Assay
Q	Quadropole
QqQ	Triple Quadrupole Analyser
SAC	Spectral Absorption Coefficient
SCADA	Data Acquisition and Data Analysis
SDBS	Sodium Dodecylbenzene Sulphonate
SERS	Surface Enhanced Raman Spectroscopy
SGAB	Sociedad General de Aguas de Barcelona
SPC	Sulfophenylcarboxylates
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
SSD	Species Sensitivity Distribution
SWRO	Seawater Reverse Osmosis Desalination Plant
TDI	Tolerable Daily Intake
THM	Trihalomethane

TOC	Total Organic Carbon
TOF	Time-of- Flight Analyser
TSS	Total Suspended Solids
TWA	Time Weighted Averaged
TU	Toxicity Units
UF	Ultrafiltration
UN	United Nations
UPLC	Ultra Performance Liquid Chromatography
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
Vis	Visible
VOC	Volatile Organic Compound
VTG	Vitollegin
WFD	Water Framework Directive
WHO	World Health Organisation
WQI	Water Quality Indexes
WSP	Water Safety Plans
WWTP	Wastewater Treatment Plant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Introduction

1. Introduction

The monitoring of water bodies quality is a requirement nowadays but not only for the preservation of ecological and chemical status. Final uses of this water, especially for irrigation, industrial application or potable purposes demand high quality. Treatment technologies are implemented when quality does not meet requirements for these specific uses, but the performance of these technologies and the quality of produced water are also very dependent on the characteristics of the resource at the intake.

Several strategies can be applied for the monitoring of these waters, including the classical sampling and transport to the laboratory in order to perform analysis, the use of kits and portable devices, and the installation of on-site instruments that are able to operate automatically and communicate remotely.

There is no single optimal strategy to follow for the monitoring of the quality of waters. This depends on a set of factors: the paremetres to be monitored (ranging from physico-chemical paremetres such as conductivity to specific organic molecules or microorganisms), the final objective of the monitoring (ranging from global alert systems to the unequivocal identification and quantification of specific molecules), and other restrictions (financial resources, accessibility, etc.). In the end, it will be necessary to choose an adapted strategy for each case or a combination of several strategies.

Although monitoring of the quality of waters is performed routinely nowadays, new advances need to be made in the identification of emerging paremetres, the use of advanced technologies and their validation for different uses. To evaluate the chemical status of water, quantification of legislated paremetres may not be enough. The creation of indexes based on the toxicity of compounds for ecosystems and for the public may be a useful tool for this purpose.

This chapter will include an introduction to the topic of monitoring river and drinking waters plus the methodologies and technologies that will be the basis for the research that has been performed in the framework of this thesis. River waters were selected as a representation of natural water resources as these waters are the main source of drinking water production in the case where the research was performed: the Barcelona area and the Llobregat River (NE Spain). Therefore, Llobregat River is considered not only as an aquatic ecosystem itself but also as a source of drinking water.

1.1. General Framework: the monitoring of water quality

Traditionally, the principal reason for monitoring water quality has been the need to verify whether observed water quality is suitable for intended uses. Monitoring has evolved over time and the main purposes may be to (adapted from the World Meteorological Organisation (2013):

- Support decision-making and operational water management in critical situations. When pollution events occur, reliable data are needed, which may require early warning systems to signal when critical pollution levels are exceeded or toxic effects occur;
- Determine trends in the quality of the aquatic environment and how the environment is affected by the release of contaminants, often known as "impact monitoring";
- Determine treatment options for polluted or undrinkable water;
- Determine ecological flows, that is,the flow regime required in a river to achieve desired ecological objectives;
- Evaluate the effectiveness of water management measures;
- Provide the basis for the formulation of science-based environmental policies;
- Evaluate water-quality trend over a period of time, and understand the environmental fate of different pollutants;

Although the impact of pollution on surface waters in Europe is being continuously reduced, probably due to the reduction of industrial toxic discharges because of the implementation of stricter governmental regulations and the development of cleaner technologies, complementary improvements in the elimination of priority pollutants, the control of discharges and environmental monitoring should be achieved.

The European Commission's Joint Research Centre (JRC) identified water quality and availability as issues that are evolving at a critical pace. Table 1.1 summarises the key issues recommended to protect water resources, reported in the JRC`s strategic paper "The Water Challenge" (Joint Research Centre, 2000).

Table 1.1 Issues, approaches, and specific actions needed to safeguard water resources (adapted from "The Water Challenge")

Issue	Approach	Specific Actions
Ensure that demand for water is matched by availability	Integrated water management	Develop measures for groundwater management rehabilitation.
	Reduction in surface and groundwater pollution	Develop measures to promote effective water use, with an emphasis on demand-side management. Develop economic and fiscal measures. Study the quality of water resources regarding present/futures uses.
Maintain and improve groundwater quality	Prevention of point pollution and reducing non-point pollution	Define chemicals and good quantitative status of groundwater.
Maintain a high standard of ecological quality of surface water biodiversity	Development of new directives embracing ecological principles	Establish operational indicators of ecological quality for surface waters. Measure and identify long-term trends in eutrophication and acidification of Europe's water. Improve awareness about biological and ecological effects. Implement European water resources monitoring network. Define the data needs for the development of integrated environmental assessments on specific issues (e.g. eutrophication).

1.1.1. Legislation

Legislation aimed at improving water quality is being developed on the basis of lists of pollutants used in industrial processes or products, such as pesticides, applied in the environment. Many of these are known or are suspected to produce significant impacts on the environment and human health. Some of these compounds persist in the environment and bio-accumulate, having potential negative effects on organisms (e.g. endocrine disruption). These lists currently form the basis of risk assessments and related management plans.

At a European level, quality fulfilments to be met are mainly addressed by Water Framework Directive (WFD, 2000/60/EC) and the updated Priority Substances Directive (2013/39/EU) where Environmental Quality Standards (EQS) such as Maximum Allowable Concentration (MAC) and Annual Average (AA), are established for a list of 45 pollutants in surface waters and biota. The WFD defines EQS as concentrations of pollutants in water, sediment or biota that should not be exceeded in order to protect the environment, although nowadays only EQS has been established for surface waters. The WFD has led to a critical assessment of existing monitoring practices, and, in most river basins, this has required an upgrading of monitoring systems.

Performing laboratory analysis of the list of 45 pollutants implies a large number of analyses. Additionally, it should be taken into account that it is not enough to measure whether or not the priority substances' concentration is below the EQS as this is not necessarily representative of the water status. Moreover, spot sampling campaigns, the most common approach for analysing these compounds, are costly and labour-intensive and not sufficient to ascertain an accurate picture of the chemical and biological status of water quality on a yearly basis.

Linked to the WFD, other Directives exist in the field of water quality:

- Legislation concerning the protection of groundwater (2006/118/EC)
- Strategies against chemical pollution of surface water, such as the legislation on the Discharges of Dangerous Substances Directive (76/464/EEC)
- Water pollution coming from urban wastewater and certain industrial sectors regulated by the Urban Waste Water Treatment Directive (91/271/EEC)
- The quality of bathing waters in rivers, lakes and coastal waters regulated by the Bathing Water Quality Directive (2006/7/EC)

Additionally, the approval of the Environmental Liability Directive (2004/35/EC), concerning the prevention and mitigation of environmental damage, poses the principle of prevention and repair on the basis of the "polluter-payer" principle of damage to the environment caused by an operator (the damages concerned are those severely affecting soil status, ecological, chemical or quantitative status or the ecological potential of water, wild species of fauna and flora and protected natural habitats).

In tandem with these initiatives, the Commission approved a regulatory framework for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). It entered into force as Regulation 1907/2006. It streamlines and improves the former legislative framework on chemicals of the European Union.

The main aims of REACH are to ensure a high level of protection of human health and the environment from the risks that can be posed by chemicals, the promotion of alternative test methods, the free circulation of substances on the internal market and enhancing competitiveness and innovation Additionally, the INSPIRE Directive (2007/2/EC) establishes an infrastructure for spatial information in the European Community to support environmental policies and policies or activities which may have an impact on the environment.

Concerning other policies beyond legislation, the European Union Water Initiative was launched to contribute to the achievement of the United Nations Millennium Development Goals with regard to drinking water and sanitation targets. The UN World Water Assessment Programme seeks to develop the tools and skills needed to achieve a better understanding of those basic processes, management practices and policies that will help improve the supply and quality of global freshwater resources.

One of the most sensitive uses of natural waters is their treatment for drinking purposes. Source water must comply with the requirements that make it suitable for its treatment in drinking water plant facilities. European and national legislation have also been established to protect drinking water consumers. The Drinking Water Directive(98/83/EC) and the application of Water Safety Plans (WHO Guidelines and ISO 22000:2005) impose strict regulations on the monitoring of water quality indicators, mainly focused on protecting human health. Moreover, treatment works have been designed for removing certain compounds at a range of concentration. An unexpected alteration ofthe quality of water could impact on the efficiency of the processes. Therefore, regulatory and technological requirements make it necessary to monitor water at the entrance of treatment plants.

1.1.2. Global approaches to the monitoring of water quality

The methods used to monitor pollutants of concern very commonly involve taking volumes of water in glass or plastic containers at specified periods of time from the field to the laboratory where they are analysed. In most cases, accredited methods are used to analyse different types of substances, and there is a high degree of confidence in the results obtained. However, there is less confidence in the sampling procedures, since low-frequency sampling at a restricted number of sampling points may not provide a representative picture of water quality, because pollutants levels vary both spatially and temporally. Furthermore, the levels of substances measured in the laboratory may not reflect the bio-available fraction present in the water (de la Cal et al., 2008). In some cases, the sample preparation (e.g. different degrees of filtration) can affect the material available for quantification. Many pollutants can relate to some extent to other compounds in the water sample, such as suspended particulate matter, and dissolved large molecular weight organic compounds (e.g. humic and fulvic substances) (Dworak et al., 2005; Graveline et al., 2010).

Therefore, there is a need to use some alternative monitoring tools to complement or even partially replace the traditional ones mentioned above. Some provide rapid, in-situ or on-site measurements, whilst others still require the collection of spot samples and their transport to a laboratory for analysis. New techniques, with lower monitoring and analysis costs compared to frequent spot sampling, could be used

to provide a comprehensive overview of water quality on a river basin scale and to offer a rigorous basis for the risk assessments and subsequent decisions on corrective actions (Geiszinger et al., 2009).

An alternative way to complement the analytical determination of pollutants in grab samples is the use of innovative in-line systems that pre-concentrate pollutants in water samples and offer information on a time-average basis (Santiago Sánchez et al., 2014). These devices report the average concentration during sampling time. Although not providing information on peak pollution, they reflect pollution episodes that could have taken place between individual grab samples. Another advantage is the possibility of detecting pollutants below the detection limit of the analytical equipment as they are being accumulated in the sampling device (Kot-Wasik et al., 2007). The application of these tools for water quality monitoring could mean a great advantage considering that some legislation (2013/39/EU) demands the reporting of annual averages and not just peak concentrations.

Due to instrumentation development in the field of analytical chemistry, it has been possible to identify and quantify a large range of pollutants in all biological or physical matrices. This information warns of the presence of these contaminants in the ecosystems, but their ecotoxicity is not taken into account. Accordin to Bogue (2008), the environmental protection agencies routinely analyse water samples for around 200 different compounds plus other variables that give a broad indication of the health of the aquatic environment (e.g. pH, dissolved oxygen, toxicity, conductivity and suspended solids). In monitoring pollutants, difficulties arise because of: the great number of compounds involved, many of which need to be determined at trace levels; many sources are diffuse rather than point; and many different aquatic matrices and environments are subject to pollution (rivers, lakes, aquifers, wetlands, reservoirs, drinking water, seas and oceans). Some of the most significant water pollutants and their sources and effects according to Bogue (2008) are listed in Table 1.2.

Biomonitoring is used as a complement to physical and chemical determinations of water quality. This involves evaluating a disturbance to the environmental status by using the properties of certain living organisms that will react to these disturbances. The biological and ecological response of target species provide information on the presence, type and sometimes estimated quantity of pollutants in an ecosystem. This is a valuable assessment tool receiving increasing interest and being used progressively in different types of water quality monitoring programmes (Allan et al., 2006; Malhotra et al., 2005).

Table 1.2. Aquatic pollutants, sources and impact (Bogue, 2008)

Pollutant	Sources	Impact
Ammonia	Sewage treatment, industry, agriculture	Toxic to fish and aquatic invertebrates
Nitrates	Agriculture, industry, bacterial oxidation of ammonia	Eutrophication, possible threat to human health
Phosphates	Industry, sewage treatment, agriculture	Eutrophication, toxic to aquatic wildlife
Chlorine	Drinking water treatment, industry	Aids formation of trihalomethanes, toxic to fish and aquatic invertebrates
Trihalomethanes	Reaction between chlorine and organic compounds	Possible human toxicity, imparts antiseptic taste to drinking water
Pesticides and herbicides	Agriculture, industry, agrochemical production	Toxic to aquatic wildlife, possible human toxicity, inhibition of water treatment processes
Fuels and oils	Spillage and leakage, run-off, industry, discharges from oil tankers	Death of seabirds and marine life, contaminated beaches, depletion of fish stocks, possible human toxicity, inhibition of water treatment
Solvents	Run-off from land, industry, landfills	Toxic to aquatic wildlife, contaminated aquifers, toxic/carcinogenetic to humans
Phenols	Sewage treatment, industry, agriculture, landfills	Toxic to aquatic wildlife, possible human toxicity, impart unpleasant taste to drinking water
Metals	Industry, mining, landfills, run-off	Toxic to aquatic wildlife, human toxicity, inhibition of water treatment, bioaccumulation by marine life
SS/turbidity	Industry, sewage treatment	High BOD, settling can damage aquatic ecosystems
Bacteria, viruses, other	Sewage treatment, natural sources	Infections and fatalities in humans and livestock
Radioisotopes	Industry, healthcare, mining, natural sources	Fatal and non-fatal cancers in humans

Table 1.3. Water Quality Sensing Technique (Bogue, 2008)

Compound	Techniques
Ammonia, phosphates	ISE, colour chemistry plus photometry, auto titration
Nitrates	ISE, UV absorption, colour chemistry plus photometry
Metals	Voltammetry, colour chemistry plus photometry
Oils/fuels/solvents	UV fluorescence, UV photometry, electromagnetic absorption, optical scatter and reflection, capacitive, vapour purging plus VOC gas sensor
BOD	Bacterial biosensor, biomass oxygen consumption
COD	Thermal/chemical oxidation plus IR-based CO2 detection, ozone oxidation/consumption, UV/visible spectrometry (inferential method)
TOC	Oxidation plus IR-based CO2 detection, UV/visible spectrometry (inferential)
Toxicity	Bacterial oxygen consumption, algal fluorescence, microbial respiration inhibition
DO	Clark electrode, fluorescence quenching
SS/turbidity	IR and visible light scatter, optical absorption
Conductivity	Current flow between two electrodes
Total ion concentration/TDS	Conductivity sensor
pH	Glass electrode
Algae/chlorophyll	UV fluorescence

Notes: ISE – ion-selective electrode; TOC – total organic carbon; TDS – total dissolved solids

Biomonitoring uses sentinel species, defined as any living organism to be used as an indicator of the presence of a pollutant or the toxicity of a contaminant (Amiard and Amiard-Triquet, 2008). Sentinel species can be:

- Bioindicators: species used in the absence or presence criteria, and/or abundance criteria
- Bioaccumulators: species that have the capacity to accumulate contaminants present in the environment
- Species that has modifications at molecular, cellular, physiological, organic or behavioural levels, which can be used to assess the risks associated with presence of a pollutant (biomarker and bioassay)

For the implementation of WFD, studies at a community level (bioindicators) appear suitable for assessing the ecological quality of water bodies, whereas the bioassays/biomarkers are especially useful as early warning systems and to investigate the causes of ecological impairment, thereby enabling a better understanding of the cause–effect-relationships (Martinez-Haro et al., 2015).

In the past, regulatory changes tended to focus on a continual decrease in allowable analyte concentrations, relating to the development of enhanced laboratory instrument sensitivities. Future regulations may well focus on the consistency of water quality, by exploiting the capabilities of online technology. Future regulatory control strategies might be based on the percentage of time in compliance rather than on instantaneous concentrations not to be exceeded. Requirements and time periods to perform the measurements would be established by following a risk analysis (AWWA, 2002).

Concerning water quality sensors, they use a range of electrochemical, optical and biochemical techniques (Table 1.3) and several are effectively automated versions of the standard laboratory methods. Academic and industrial research groups are working on the development of new and improved environmental sensors, which include (Bogue, 2008):

- A desire to replace some laboratory determinations with measurements in the field or at the point of discharge;
- A need for more sensitive sensors in response to falling emission limits and other standards; and
- A frequent requirement for more data to provide improved spatial coverage.

The first of these reflects both cost and operational considerations and also the benefits arising from the ability to obtain data more rapidly. This is particularly important when investigating water pollution incidents and also where sites that are being investigated are remote from analytical laboratories.

1.2. Case study: Barcelona Metropolitan Area and the Llobregat River basin

The Llobregat River emerges at Castellar de n'Hug, North West of Catalonia, Spain (see Figure 1.1), at an altitude of 1400 m and flows approximately 160 km, discharging its waters into the Mediterranean Sea, 10 km south of Barcelona. The Cardener and Anoia Rivers are the main tributaries.

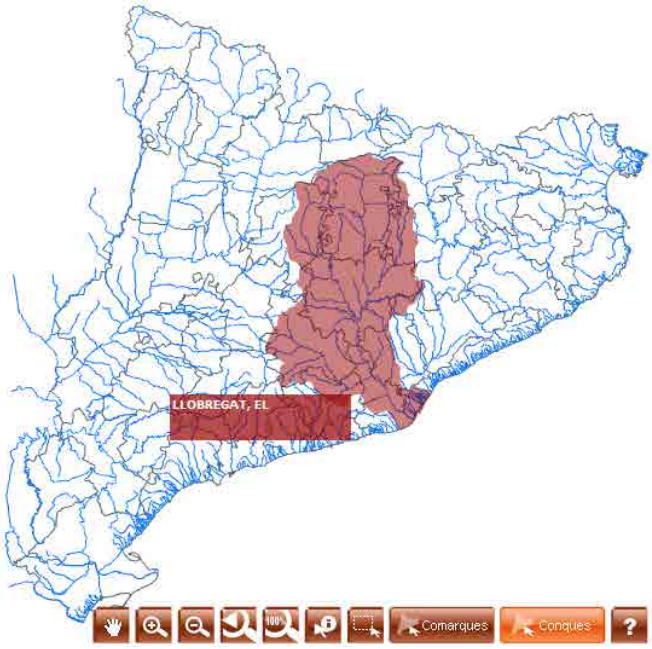

Figure 1.1. Screenshot of the Llobregat River basin highlighted in red on a map of Catalonia (http://aca-web.gencat.cat/sdim/visor.do)

As a Mediterranean river, is highly dependent on climatic conditions and the flow can range from over 600 $m3s^{-1}$ in the stormy seasons (spring and autumn) to a few $m3s^{-1}$ during the dry season (summer) leading to worse water quality due to the increase of effluent wastewaters in the total flow of the river (Mosley, 2015).

This river is also characterised by its salinity from salt mines located in the upper course of the river and from the geological formation present in the basin. Although in the 1990s a brine-pipe was built with the aim of collecting the mining lixiviates, the salinity problems in the Llobregat River Basin were not totally solved and high salinity levels are still found in some stretches of the river. In the lower-middle course of the Llobregat and Cardener Rivers there is a large concentration of industries, agricultural activities and densely populated areas with significant demands of water. The Anoia River is mainly influenced by the agricultural areas (vineyards) and industries.

More than 30 Wastewater Treatment Plants (WWTPs) have been set up in order to improve the water quality of the Llobregat River and its tributaries, treating a mixture of urban and industrial wastewaters. The main industries sited along the Llobregat River are tannery, food products, textile, and pulp and paper industries discharging a broad spectrum of organic chemicals into the river. Therefore, the river receives effluents from these WWTPs and surface runoff from agricultural areas.

A high contribution of treated wastewaters discharges into the total flow of the river is expected as the low flow makes the dilution factor almost negligible. The removal of contaminants by the WWTPs is not always complete; consequently these pollutants can enter the environment via sewage effluents and thus become a potential risk to the receiving bodies and, in addition, to the production of drinking water (Gasperi et al., 2008; Muñoz et al., 2009; Collado et al., 2014). At rainy periods, a higher and more turbulent flow implies a resuspension of the sediments from the riverbed. In industrial areas, polluted sediments could also pose a threat to water quality. An additional threat to water quality at rainy periods comes from the fact that an overflow of the sewerage network could take place, causing a direct discharge of wastewaters into the river.

The Llobregat River is one of the main drinking water sources in the Barcelona Metropolitan Area (BMA) due to the scarcity of groundwater resources. Therefore, the quality of the raw water must be controlled and consequently a group of surface water quality control stations was set up in recent decades providing data about general paremetres such as turbidity, pH, conductivity, dissolved oxygen, temperature, total and dissolved organic carbon, ammonia, phosphates, nitrates and a selection of heavy metals. Previous studies and monitoring campaigns show the presence of a significant number of families of pollutants (Céspedes et al., 2005; González et al., 2012; Kuster et al., 2008). Also, in order to enhance the water quality of the river, several by-passes have been constructed along the river, thereby stopping the most contaminated discharges from reaching the river before the drinking water treatment plants (DWTPs).

Abrera DWTP and Sant Joan Despí DWTP, located in the lower-middle course of the Llobregat River, supply approximately 40% of drinking water to the Barcelona metropolitan area and surrounding area, other sources being the Ter River, El Prat desalination plant and groundwater. In order to adapt the DWTPs to the European legislation requirements (some parametric values were updated in 2008, 10 years after the entry into force of Directive 98/83/EC), both DWTPs have implemented new infrastructures in recent years (Figure 1.2).

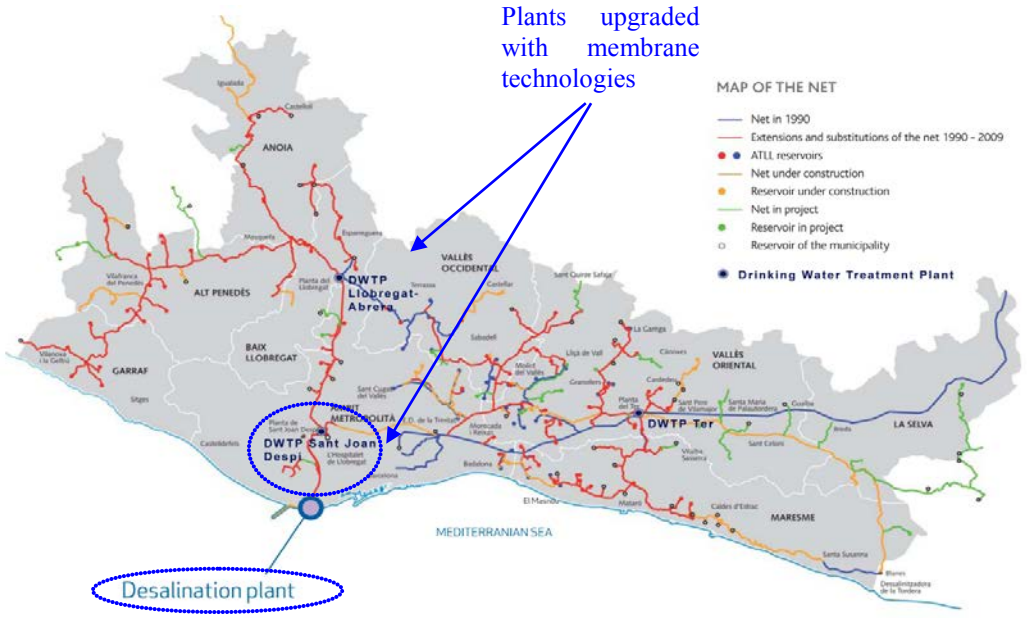

Figure 1.2. New drinking water treatment facilities in the Barcelona area (source: www.atll.com)

Sant Joan Despí DWTP opted for an additional ultrafiltration and reverse osmosis treatments, thereby reducing the presence of target compounds and treating 50% of the effluent. In contrast, Abrera DWTP installed the world's largest electrodialysis-reversal (EDR) desalination plant, which can remove up to 154 tons of salt water per day. Both plants provide a reduction of the formation of trihalomethanes in drinking water to levels below 100 µg/L as requested in the Drinking Water Directive (98/83/EC)(Valero and Arbós, 2010).

1.3. Need to monitor surface water

The management of rivers and the wider hydrological environment of a river basin also influence ecological and water quality. Over time, with the advent of industrialisation and increasing populations, the range of requirements for water has increased, together with greater demands for higher-quality water, such as for drinking and personal hygiene, fisheries, agriculture (irrigation and livestock supply), navigation for the transport of goods, industrial production, cooling in fossil fuel (and later also in nuclear) power plants, hydropower generation, heat/cold storage in aquifers, recreational activities such as bathing or fishing and nature conservation, e.g. wetlands. Each water use, including abstraction of water and discharge of waste, leads, however, to specific and generally rather predictable impacts on the quality of the aquatic environment.

The high variability of sources of pollution means a large number of compounds and paremetres need to be measured, from the basic physical-chemical paremetres like temperature, turbidity, salinity, organic matter or pH to simple compounds that could be present in water having a known effect on ecosystems like ammonia, nitrate, phosphate, heavy metals or more complex organic compounds included in regulations like pesticides, alkylphenols, etc. There are also some other pollutants that could cause a risk to health or the environment but whose effects are not completely studied and reported on yet, which are the emerging compounds such as hormones, antibiotics, surfactants, endocrine disruptors, human and veterinary pharmaceuticals, X-ray contrast media, pesticides and metabolites, disinfection by-products, algal toxins and taste-and-odour compounds (Pal et al., 2014).

There are numerous classifications of the contaminants that can be found in surface waters. Bartram and Ballance (1996) presented the following classification:

- Faecal contamination from sewage makes water unsafe for human consumption and aesthetically unpleasant and unsafe for recreational activities. Many organic pollutants consume oxygen, suffocating fish and other aquatic life.
- Nutrients, such as nitrates and phosphates, from farm fertilisers to household detergents cause the growth of large mats of algae, some of which can be toxic. When the algae die they decompose, consume oxygen and damage ecosystems.
- Pesticides and veterinary medicines from farmland and some industrial chemicals can threaten wildlife and human health. Some of these damage the hormonal systems of fish, causing “feminisation” (endocrine disruption).
- Metals, such as zinc, lead, chromium, mercury and cadmium, are extremely toxic. Copper complexes are less toxic, and cobalt and ferrous complexes are only weak toxicants.
- Organic micropollutants, such as pharmaceuticals, hormones and chemical substances used in products and households, can also threaten health.
- Chlorinated hydrocarbons exist in the natural systems and several are highly toxic for humans. These molecules persist in the environment for a longer time and threaten to contaminate aquatic and soil systems.
- Sediment runoff from the land can make water muddy, blocking sunlight and, as a result, kill aquatic life. Irrigation, especially when used improperly, can bring flows of salts, nutrients and other pollutants from soils into water.

Good knowledge about the occurrence of these compounds and their effect on living organisms, including human beings, are of major importance to performing a risk assessment related to their presence in surface waters. This means that not only quantification of these pollutants is important, but also it is

evaluating their toxicity in order to develop indicators that could assign the proper weights to compounds detected according to their harmfulness. Another field of research is the study of the behaviour of pollutants during water treatment and in the environment. It is important to detect not only parent compounds but their degradation products as they could be even more toxic than the former ones (e.g. nonylphenol exhibits higher toxicity than the parent compound nonylphenol ethoxylates used as a non-ionic surfactant) (González et al., 2007).

The key to continual improvements in environmental quality is the availability of precise and representative information of the quality of water bodies. Reliance on infrequent spot sampling is unlikely to fulfil this increasing need. A range of alternative tools are emerging and although they have not shown the same low level of uncertainty that is now taken for granted in classical analytical methods yet, they can provide more representative data, often at a lower cost. These include sensors, field test kits, passive samplers, biological early warning systems, biomarkers, and ecological indices. It is important that further work is carried out to demonstrate the utility and reliability of these emerging tools in laboratory and field trials compared to classical methods. This will be necessary to provide a wide range of well-defined tools to make it possible to select the most appropriate solution for each specific application.

Different techniques could be used to monitor water quality depending on the information that needs to be obtained. A classification of these techniques has been included as Table 1.4. When designing a strategy for monitoring the water quality of natural waters, a combination of them should be considered depending on the specific needs. For the classification of techniques, the following aspects are taking into account (Allan et al., 2006):

- Paremetres to be measured. Different techniques have been designed for the identification of different paremetres. Physicochemical properties are inherent to the whole mass of water and a large number of probes have been designed over the years, based on different technologies, so a rapid measure can be obtained on site. In contrast, some organic compounds are present at such low levels (pg/L) that sophisticated equipment is necessary to detect them after a sample pre-treatment at the laboratory, implying costly and elaborated methods. Technology should be chosen according to the properties of the target compound.

- Application field. Information on water quality could be obtained for different purposes. A study on the impact on river ecosystems could involve biological sensing techniques that will mimic the effects of water quality on living organisms. When the purpose is to detect alteration in water quality to protect intake of DWTPs, systems providing information in real time are needed. When monitoring a specific family of compounds at trace levels and its behaviour, advanced techniques

at laboratory are used. These techniques require a sample concentration that could be done in the laboratory in spot sampling or on site when passive samplers are implemented to obtain average information over a period of time.

- Cost. Information on the cost of acquiring technologies, their maintenance, staff requirements, reagents, etc. needs to be assessed when performing the analysis.

In situ and portable water quality monitoring devices are often used, as there is an increasing need to monitor large areas in short time periods. Field test kits can accomplish the initial screening and periodical monitoring of waters. Such tests are relatively inexpensive and can be conducted at water-user level. The ability of a portable monitoring device to provide an immediate result has its application in isolated and developing areas where the safety of drinking-water supplies is of paramount concern.

In-situ sensors enable the continuous or intermittent collection of water quality paremetre data in real or near-real-time. High density of measurements over relatively short periods can be critical because water-quality conditions can vary widely, such as before, during and after storms. Sensors can be cost effective because they minimise costly field visits by scientists and technicians. The monitoring devices may also include flow-through systems that enable rapid measurements, either as continuous stationary measurements or used on a moving platform (boat). It should be stressed, however, that the use of these special, multi-parametric, portable instruments and automatic water quality stations still require regular (and sometimes sophisticated and expensive) maintenance. Advances related to monitoring technology are needed to support future water-quality issues successfully. They include, for example, continued development and testing of probes, monitors, data-recorders and telemetry equipment that allow the monitoring of water-quality variables (WMO, 2013).

When data is obtained, especially in the case of sensors generating a huge amount of data, an analysis should be done to extrapolate the information needed. Strategies could involve obtaining data at the same site at different times to study evolution; at different sites at the same time, as a monitoring network; different sites at different times, understood as the evolution of a network needed for providing data for models; or even different data at the same site, requiring multiparametric sensors or a battery of different sensors or analysis (UKWIR, 2008). After the analysis is done, the information can be compared to field ecological observations and can be used to provide indicators for the ecological status of water bodies, develop river basin management plans or provide early warning strategies.

Table 1.4. Characteristics of the main types of tools and technologies for chemical and biological monitoring requirements within the WFD (Allan et al., 2006)

Tools	Principle	Value measured	Deployment characteristics	Applicability	Advantages	Drawbacks
Water quality evaluation software	Assessing water quality based on physico-chemical measurements and benthic fauna assemblages and composition	Deviation from expected pristine condition for specific conditions of a particular site	Spot sampling followed by laboratory analysis	Freshwaters, rivers and lakes/estuaries/sea waters		
Biomarkers	Any biological response to an environmental chemical(s) at the sub-individual level, measured within an organism and its products	Chemical or pollutant concentrations	Spot sampling followed by laboratory analysis	Most types of waters	Early detection of contaminant impact and interaction with receptor organism	Need to account for the influence of their biological function
	Indicators of toxicity, exposure and susceptibility	Physiological and biochemical alterations specific to classes of pollutants		Many pollutants		Sometimes, need comparison to reference site
Whole-organism bioassays	Test based on the reaction of whole-organisms to toxicants present in water samples	Acute toxicity (including. geno-toxicity, cyto-toxicity or mutagenicity)	Laboratory and spot sampling based assays (a few in-situ methods)	Most types of waters including groundwater	Very useful as preliminary screening devices May be combined with toxicity directed analysis schemes	Only provide information on the acute toxicity of samples Results after 24–72 h
Biological early warning systems	Whole-organism bioassay specifically adapted to real-time measurements based on behavioural changes	Acute toxicity	On-line, in situ at secured sites	Most types of waters Monitoring at remediation sites	Use of different trophic levels	Need energy supply Fails to provide longer term toxicity information
Spot sampling + chemical analysis	Collection of a water sample followed by extraction/filtration and chemical analysis (GC, ICP–MS)	Total contaminant concentrations	Bottle sampling	All types of waters Most chemicals	Easy to defend in court Accuracy may be determined relatively easily	Labour-intensive Provide a snapshot of the situation at sampling time Does not account for bioavailability

Table 1.4. Characteristics of the main types of tools and technologies for chemical and biological monitoring requirements within the WFD (Allan et al., 2006) (cont.)

Continuous monitoring	Chemical analysis of continuous on-line water or 24 h composite samples	Pollutant concentrations	On-line at secured sites	Many organic pollutants	Rapid warning of concentrations exceeding EQSs	Need power supply and laboratory set-up at secured site
Passive samplers	Bio-mimetic sampling to mimic bioaccumulation or based on contaminant diffusion-limited accumulation into samplers	Bioaccumulation in aquatic organisms or truly dissolved time-averaged pollutant concentrations	In-situ deployment at secured/unsecured sites and laboratory analysis	Most types of waters	Needs no energy supply	Bio-fouling problems
				Priority pollutants (inc. polar/non-polar organics metals and heavy metals Most types of waters	Deployment times from days to months Suitable for most types of waters Inexpensive	Need for extensive laboratory calibration
Biosensors	Analytical device incorporating a combination of a specific biological element (creating a recognition event) and a physical element (transducing this event)	Total and bio-available pollutant concentrations General toxicity, geno-toxicity and cyto-toxicity measures (BOD)	In situ, laboratory-based and continuous monitoring	Priority pollutants Organic and inorganic pollutants	May be based on continuous and on-site monitoring	Often requires skilled operators Not applicable to all pollutants
Sensors	Detection and quantitation based on physico-chemical characteristics of contaminants	Contaminant concentrations	In situ, laboratory-based and continuous monitoring	Most types of waters Heavy metals, PAHs, and certain pesticides	Handheld instruments	Not applicable to all pollutants
Immunoassay test kits	Highly selective pollutant extraction and/or quantitation based on antigen/antibody interactions	Pollutant concentrations	Field or laboratory assays based on spot-sampling	Many organic pollutants e.g. pesticides, PAHs	Rapid and easy to employ	Unit: analyte equivalents
				Certain metals	Very sensitive, selective, rapid and inexpensive assays Easy to employ Ability to process many samples	Cross-reactivity with analogues and metabolites False positives Positive results may require further analysis

1.4. Need to monitor drinking water

Drinking water quality is affected by the characteristics of water at source, treatment technologies and interaction with pipe materials, among other factors. The monitoring of water quality in the drinking water supply system is very important for management purposes, as it provides information not only on the safety of the water for its potable use, but on the current blend of waters coming from different sources, the technologies that have been used and the composition of pipe materials. The interactions between pipes and drinking water affect not only the quality of waters through the leaching of pipe material but by producing corrosion on pipes. These interactions can be altered if water quality is modified.

Online drinking water quality monitoring technologies have made significant progress for source water surveillance and water treatment plant operation. The use of these technologies in the distribution system has not been beneficial due to the high costs associated with installation, maintenance, and calibration of a large distributed array of monitoring sensors. This has led to a search for newer technologies that can be economically deployed on a large scale (Banna et al., 2014).

The same technologies used for surface water monitoring can be adapted for their use in drinking water. Although the matrix is less complex than surface waters, the pollutants are found in much lower concentrations. Systems for monitoring drinking water need to be much more sensitive. Additionally, level requests are more stringent as this water impacts directly on the health of water consumers.

Threats such as terrorism attacks or accidental pollution episodes pose new challenges even to efficient operators in developed countries. Preparedness is crucial for facing such new threats, but new methodologies for detecting incidents are unquestionably needed, as well as new management strategies based on risk assessment protocols. The traditional strategy of grab sampling plus laboratory analysis is not reliable enough to protect the public from accidental o deliberate contamination. Monitoring programmes based on the analysis of certain compounds at the laboratory are thought to control mid or long-term deviation of the paremetres but not sudden changes, although nowadays this is the only strategy able to identify unequivocally and quantify precisely single molecules.

According to the vision of the European of Security Research and Innovation Forum (ESRIF, 2009), most households will have intelligent water meters installed that will be equipped with low-cost miniaturised sensors, automatic valves and communication devices for readout and remote control. Real time hydraulic and water quality models will be able to accurately predict the water quality at the point of these sensors, based on the automatic meter reading and upstream water quality. Model predictions can be compared

with current sensor readings and in the event of any deviation results may be used to trigger an alarm. However, the existing systems (networks with a low density of sensors and presenting deviations between predicted and measured water quality) are not robust enough to trigger an alert.

Based on the type of alert, the structure designed for both water utilities and local authorities should provide water supply managers with reliable tools, Early Warning Systems (EWS), to efficiently give solutions to the needs of detection and assessment of chemical, biological, radiological and nuclear (CBRN) events.

Once target contaminants for the EWS have been identified and the range of concentration necessary to detect them has been established, it is necessary to select a monitoring technology for the particular contaminant or class of contaminants. The technology considered for use in an EWS should be evaluated to ensure that all steps of the methodology perform correctly and can detect the target contaminants without excessive interference. The data quality objectives should be defined during the design of the EWS and include specificity, sensitivity, accuracy, precision, and recovery, as well as rates of false positives and negatives. According to the USEPA (2005), these are the steps to be followed to implement an EWS in drinking water networks:

- Determine Alarm Levels. The basis for setting alarm levels will depend on the previously determined levels at which contaminants need to be detected and on the type of EWS employed. Operators should be able to set threshold values so the system automatically triggers an alarm if readings move outside of the range that has been defined as safe.
- Conduct Studies on Fate and Transport Modelling of Pathogens and Chemicals. If information is available on contaminant characteristics that affect the contaminants' fate and transport, it should be factored into the design of an EWS. This information in turn can be used to select optimal locations for sensors.
- Determine Sensor Location and Density. The location and density of sensors in an EWS is dictated by the results of the system characterisation, vulnerability/threat assessment, usage considerations, risk minimisation, and cost. It may be beneficial to develop a hydraulic model of the system to assist in the placement of sensors. Real-time integrated pressure and flow data can be used to build flow models that have well characterised predictive capabilities. Table 1.5 shows the advantages and disadvantages of measuring contamination at different locations of the water distribution system.

Table 1.5. Advantages and disadvantages of monitoring at different locations of the drinking water distribution system (ASCE 2004)

Location	Advantages	Disadvantages
Source waters	Covers large segment or all of system Long lead time for response Long time for corroboration For navigable source waters threat of contamination can be relatively high To be of concern to public health, large quantities of contaminant needed—therefore easier to detect	Threat of intentional contamination of source waters is relatively low for source waters on which no commercial traffic flows
End of water transport or aqueducts	Threat of intentional contamination is slightly higher than for sources Covers large segment or all of system	Low threat for intentional contamination
Treatment plant	Threat of intentional contamination is slightly higher than for source or transport Insider threat higher	Relatively low threat for intentional contamination because access is limited and there is potential for discovery
Finished water reservoirs	Threat of intentional contamination considerably higher	There may be many of them requiring coverage
Early distribution system	Moderate threat, particularly at sites to which access can be gained (including valves, pumps and check points) Relatively long time available for warning and response	Need several platforms to cover entire system
Mid distribution system	Higher threat, covers many of the likely contamination entry points including valves, pumps and inspection ports	Need multiple platforms to get full coverage, moderate to little warning time
Entry pipes for likely targeted customers	Higher risk area; expect better cooperation from such customers	Locating so close to user leaves very little time for effective response

- Select Systems for Data Management, Interpretation, and Reduction. One of the challenges of a continuous, real-time monitoring system is management of the large amounts of data that are generated. The use of data acquisition software and a central data management centre is critical. This will require that individual sensors deployed in the system be equipped with transmitters, modems, direct wire, or some other means to communicate the data to the acquisition and management systems. Furthermore, the data management system should be capable of performing some level of data analysis and trending in order to assess whether an alarm level has been exceeded. At a minimum, the system should notify operators, public health agencies, and/or emergency response officials.

- Establish Response Communication Links, Notification, and Decision Making. An integrated EWS would also include network and communication links between command centre operators and any people designated as response decision makers. Monitoring devices may report data to a command centre that will relay information to utility decision makers. In turn, the response may use the same secure communications links to take actions in the distribution system (e.g. shut isolation valves). Further actions may include monitoring and sampling for the contaminant at

appropriate locations in the distribution system and monitoring for surrogate paremetres that may indicate contamination (e.g., increased chlorine demand, changes in pH).

- Real-Time Data Acquisition and Data Analysis. A SCADA system (Figure 1.3) links monitoring instruments, remote telemetry units, programmable logic controllers, and a host computer in order to integrate data collection and processing into a single system-wide control centre that can be accessed from various locations (EMSOFT, 2004). Once obtained, the data can go through quality assessment and validation, aggregation, transformation, and analysis (AWWA, 2002). Data analysis is performed by specialised software.

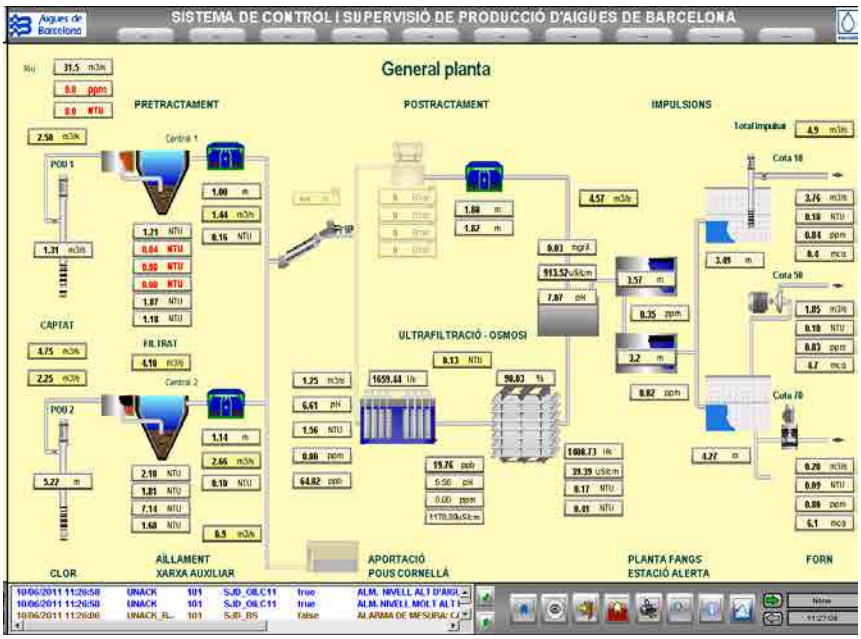

Figure 1.3. Screenshot of the SCADA system at the Sant Joan Despí Drinking Water Treatment Plant (Barcelona)

An EWS may generate real-time data for quick analysis, decisions and action. There are various techniques for assisting in real-time reporting and decision support. They include data filtering, operational indexes (commonly used by operators to calculate measures that represent trends for routine operational performance), short-term prediction using software sensors, and classification and state description to reduce information overload. Predictive modelling can also be used for assisting with data validation (AWWA, 2002).

1.5. Monitoring techniques

Monitoring is required to cover a number of water quality paremetres including physical-chemical, hydro-morphological, biological and chemical data. Chemical monitoring is expected to become more intense and toxicological tests should be included as well. The validation of cost-effective observation systems based on the integration of different sensing technologies will be the basis for chemical and biological monitoring of water quality.

Technologies applied to the monitoring of chemical quality of water bodies can be also applied to drinking waters if methodologies based on these technologies are properly adapted. Drinking water is mainly influenced by the characteristics of water in natural bodies where the intakes of the DWTPs are located. Processes during the treatment line reduce the content of organic matter and other compounds in water but the characteristics of produced water are clearly related to the water in source. Monitoring of water bodies can act as an alarm for the preserving of the intake of DWTPs and assuring a good quality of drinking waters. In contrast, characterisation of drinking water in a supply network can give information about the water quality at the source before its treatment.

The design of a monitoring programme should include: the planning of a monitoring network with the choice of location for the sampling operations, the selection of variables to monitor, the definition of sampling procedures and operations, such as in-situ measurements, manual or automated measurements, sample conservation, identification and shipment, the planning of field measurements (frequency), and the definition of the resources required (Bartram and Ballance, 1996).

When designing the programme, it should be taken into account which technology, or combination of technologies, could meet the requirements. Off-site laboratory methodology is able to identify and quantify single molecules with a high sensitivity but some variables should also be selected for early warning systems according to the availability of equipment for in-situ measurements and other cost-benefit considerations, due to the high investment, operating and maintenance costs for automatic measuring devices. Acute toxic effects may also be recognisable with the help of biological systems examining species from different trophic levels and with various functions.

An ideal integrated EWS should demonstrate a number of characteristics, such as the following (adapted from Gates (1999)):

- Provides a rapid response;
- Includes a sufficiently wide range of potential contaminants that can be detected;
- Exhibits a significant degree of automation, including automatic sample archiving;

- Allows acquisition, maintenance, and upgrades at an affordable cost;
- Requires low skill and training;
- Identifies the source of the contaminant and allows accurate prediction of the location and concentration downstream of the detection point;
- Demonstrates sufficient sensitivity to detect contaminants;
- Permits minimal false positives/false negatives;
- Exhibits robustness and ruggedness when continually operating in a water environment;
- Allows remote operation and adjustment;
- Functions continuously;
- Allows for third-party testing, evaluation, and verification.

Viable integrated EWSs that meet the desired characteristics and can be routinely used are several years away. Some individual components are available currently; however, others need further development. Most sensor and EWS components have not been third-party tested or verified, and the types of contaminants and levels of exposure have not been well defined to support selection of sensor technologies (EMSOFT, 2004).

1.5.1. Off-line methodologies for the identification of emerging compounds

In a monitoring strategy, despite the advances of real-time sensing techniques, an accurate and sensitive analysis of a large list of individual compounds can only be obtained by grab sampling and later analysis at the laboratory. This is the strategy mainly followed for the monitoring of substances that are expected to be found in very low concentrations (ngL^{-1} level) in quite stable concentrations over time.

Off-line methodologies to identify and quantify individual compounds in the laboratory are time consuming. The main constraints are related to the high investment in laboratory instruments, the cost of consumables and the need for highly-qualified personnel, especially for complex organic molecules that are difficult to analyse. Multi-residue methodologies are commonly applied to identify families of compounds having similar properties in a single analysis. These are the technologies applied for the monitoring of the occurrence of emerging compounds. As the list of compounds can be very long and their analysis is not compulsory, monitoring campaigns tend to have a low frequency.

In recent years, significant attention has been paid to the presence of pharmaceuticals and substances used in personal care products (PPCPs) in the aquatic environment. It is an obvious fact that these compounds are released into municipal sewage systems and it is also well-known that depending on their chemical structure many of them can survive the passage through sewage treatment plants or even being

transformed into more active compounds (Jelic et al., 2015). Water reclamation schemes are promoting the study of removal efficiencies and monitoring practices after tertiary treatments (Rodriguez-Mozaz et al., 2015). Research work done in recent years has resulted in refined methods for various different classes of PPCPs, in new multimethods, and in lower detection limits as well as simpler simple preparation procedures due to significantly improved mass spectrometers (Buchberger, 2011).

For the analysis of these compounds in the studies reviewed, SPE followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) is the main technique selected (Gros et al., 2006; Hernando et al., 2006; Kuster et al., 2008; Osorio et al., 2012). In recent years, the Commission Decision 2002/657/EC that was aimed at regulating the performance of analytical methods in the food industry, has also been applied to environmental analysis. According to this regulation, three identification points (IP) are needed when using LC-MS/MS for the correct confirmation of the presence of target compounds. The high sensitivity of LC-MS/MS (with triple quadrupole analysers, QqQ) makes it a very suitable, accessible technique for analysis in surface waters. The main problem is that the three IPs are not obtained for those analytes not showing two selected reaction monitoring (SRM) transitions, that is, when only one product ion can be obtained from the precursor one. This disadvantage makes this technique insufficiently reliable for the analysis of compounds such as ibuprofen or gemfibrozil.

Other techniques can be used for the analysis of pharmaceutical compounds, such as the time-of-flight (TOF) detection for the identification or complementary confirmation of these analytes. This approach has been previously tested for the analysis of pharmaceuticals but in wastewaters (Martínez Bueno et al., 2007). TOF-MS measures the accurate mass of the compounds, adding that extra point of confirmation needed to obtain a reliable result.

PPCPs, although not all are hydrophobic, can be preconcentrated by SPE. In recent years, new polymeric sorbents that improve the retention of polar compounds have become increasingly popular, such Oasis HLB, or C18 cartridges. The SPE of pharmaceuticals is generally done in an off-line mode prior to the chromatographic analysis step. Fully automated SPE procedures with single-use cartridges exist by using commercial instruments such as Prospekt-2, manufactured by Spark Holland. This robotic system employs disposable extraction cartridges (Petrovic et al., 2010).

Liquid chromatography relies on the ability to predict and reproduce with great precision competing interactions between analytes in solution (the mobile phase) being passed over a bed of packed particles (the stationary phase). The development of columns packed with a variety of materials and the systems able to precisely deliver the mobile phase has enabled LC to become one of the most used analytical techniques.

In High Performance Liquid Chromatography (HPLC), high pressure is used to generate the flow required for liquid chromatography in packed columns. Further advances in instrumentation and column technology have achieved significant increases in resolution, speed, and sensitivity. Columns with smaller particles (1.7 µm) and instrumentation designed to deliver mobile phase at 15,000 psi (1,000 bar) have come to be known as Ultra Performance Liquid Chromatography (UPLC).

After performing the separation, a mass spectrometer can measure the mass of a molecule only after it converts the molecule to a gas-phase ion. The ions are separated, detected and measured according to their mass-to-charge ratios (m/z).

The Electrospray Ionisation (ESI) is one technique for atmospheric pressure ionisation capable of creating ions at atmospheric pressure rather than in a vacuum. The sample is dissolved in a polar solvent and pumped through a stainless steel capillary, which carries between 2000 and 4000 V. The liquid aerosolises as it exits the capillary at atmospheric pressure, the desolvating droplets shedding ions that flow into the mass spectrometer, induced by the combined effects of electrostatic attraction and vacuum (as shown in Figure 1.4). A cone or counter-current gas is often applied to aid the desolvation of liquid droplets as they enter the rarified gas vacuum region of the analyser (Cole, 2000).

Electrospray ionisation is by far the most commonly used ionisation technique for the trace analysis of PPCPs in environmental samples. Unfortunately, it is prone to ionisation suppression due to matrix components coeluting with the analytes. This may lead to a loss of sensitivity and would make quantitation less reliable if external standards prepared in pure solvents were used. Various isotopically labelled pharmaceuticals have become available in recent years, which can be used as internal standards to compensate matrix effects (Buchberger, 2011).

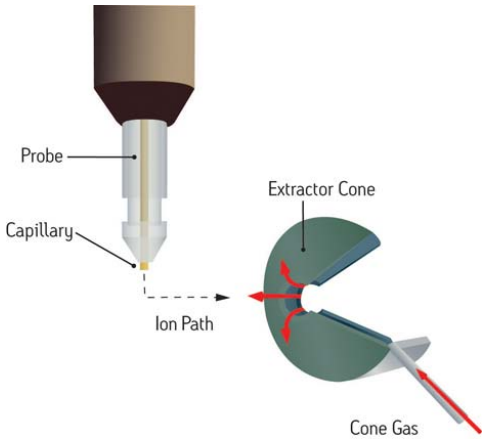

Figure 1.4. Diagram showing an ESI probe in an orthogonal position in front of the MS ion inlet

In a quadrupole (Q) mass spectrometer, superimposed radio frequency and constant direct current potentials between four parallel rods were shown to act as a mass separator, where only ions within a particular mass range, exhibiting oscillations of constant amplitude, could gather at the analyser. Systematically changing the field strength alters which m/z value is filtered or transmitted through to the detector at any given time. Single quadrupole mass spectrometers require a clean matrix to avoid the interference of unwanted ions, and they show very good sensitivity.

Single quadrupole instruments were used when the trace analysis of PPCPs began to attract increased interest, soon followed by time-of-flight (TOF) instruments. These may often still be fully sufficient for real samples, but more sophisticated MS analysers allowing MS/MS detection such as triple quadrupole (QqQ) instruments, combinations of Q and TOF (QqTOF), and combinations of Q and a linear ion trap (QqLIT) have been reported for PPCPS in a wide range of environmental samples (Petrovic et al., 2010).

In triple quadrupole (QqQ), or tandem mass spectrometers (MS/MS), there are three sets of quadrupole filters, although only the first and third function as mass analysers (Figure 1.5). The first quadrupole, acting as a mass filter, transmits and accelerates a selected ion towards the second one, which is called a collision cell. The pressure in this cell is higher, and the ions collide with neutral gas in the collision cell. The result is fragmentation by collision-induced dissociation (CID). The fragments are then accelerated into the third quadropole, another scanning mass filter, which sorts them before they enter a detector. In such an application, a precursor ion fragments into product ions, and the MS/MS instrument identifies the compound of interest by its unique constituent parts (Balogh, 2009).

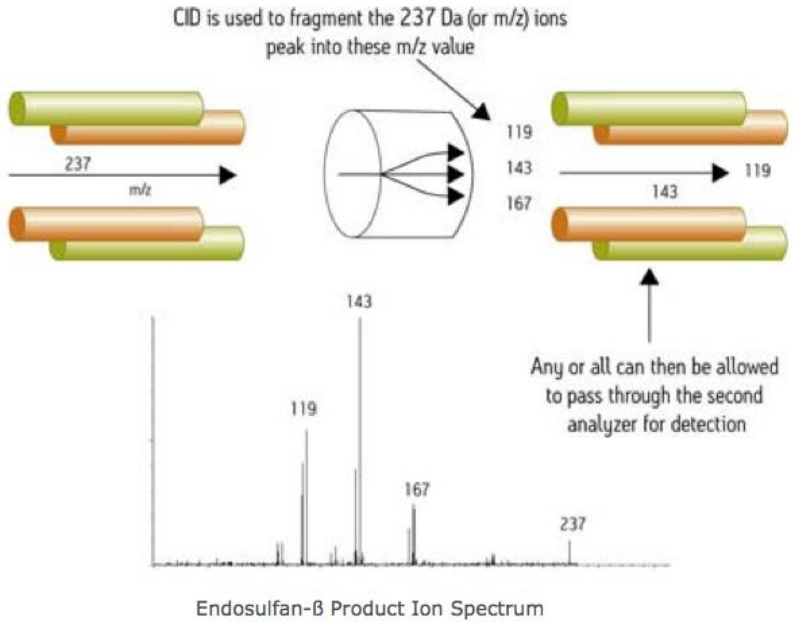

Figure. 1.5. A precursor ion entering and being fragmented in the collision cell. The spectrum displays only fragments of interest with respect to the full scan MS spectrum

A TOF instrument provides accurate mass measurement of a molecule's true mass. The TOF instrument can be used as a reflectron, aided by electrostatic grids and lenses. When operated as a reflectron, resolution is increased without dramatically losing sensitivity or needing to increase the size of the flight tube (Figure 1.6).

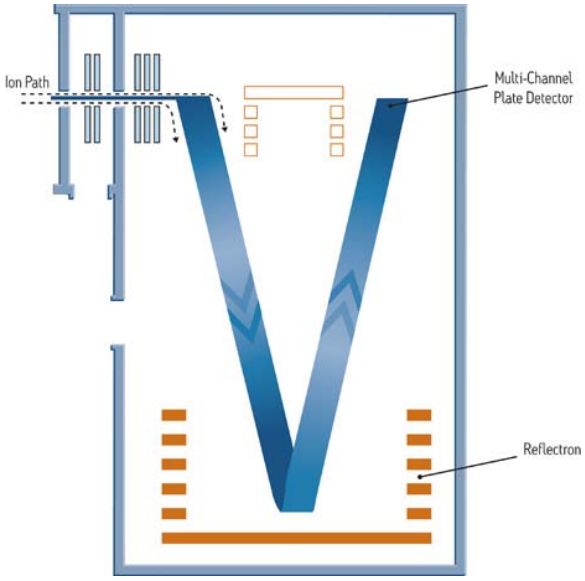

Figure 1.6. Ions are accelerated by a high voltage pulse into a drift or flight tube. Lighter ions arrive at the multi-channel plate or detector sooner than heavy ones

TOF analyses involve accelerating a group of ions to a detector. The ions exit the source having received an identical electrical charge or potential. Because all similarly charged ions share the same kinetic energy (kinetic energy = ½ mv2 where m is the ion mass and v the velocity), those with lower masses show greater velocity and a shorter interval before striking the detector. The ions travel a given distance in a time that depends on the mass-to-charge ratio (m/z). The TOF instrument can achieve a very high sensitivity relative to scanning instruments (Kanu et al., 2008).

The TOF instrument's detector offers great resolution. However, the quadrupole instrument is, generally speaking, more sensitive when detecting target analytes in complex mixtures and is, therefore, typically a better quantitation tool.

1.5.2. On-line physical-chemical probes: measurements based on UV-Vis absorption

In applications where continuous monitoring is required, UV technology is one of the sensing principles that have often been adapted for use in online instruments. The use of modern signal processing techniques, combined with state-of-the-art optics, has allowed several compounds to be detected selectively and in a reagent-free manner. Examples include nitrates, nitrites and organic load, in matrices

such as drinking water, liquid wastes and process waters (Bogue, 2008).

Most organic compounds commonly found in surface water absorb UV radiation. An analytical technique, using a UV spectrometer, is a useful alternative for measuring organic compounds in water. To estimate the concentration of organic compounds in water, UV absorption is measured at 253.7 nm (often rounded up to 254 nm). A strong correlation may exist between UV absorption and organic carbon concentration measured using other methodologies, such as TOC (ISO 8245:1999).

UV absorption is a well-defined and commonly used methodology. The light source transmits a light beam through the middle in the flow cell and the detector measures the intensity of the remaining light. After being amplified, an electric signal is delivered as the absorption reading. In a single-beam spectrometer, many variables in addition to the sample in the flow cell affect the amount of UV light that hits the detector. The luminosity of the light source and the sensitivity of the photodetector will also affect the reading. Light source fluctuations are reduced by using "dual-beam" light configurations. With this configuration, a semi-transparent mirror produces two beams: the measurement beam, which passes through the sample and strikes the first photodetector, and the reference beam, which goes straight to the second photodetector. However, the changing sensitivity of the two photodetectors remains a source of error. The optimal solution is the use of two beams and just one photodetector as shown in Figure 1.7.

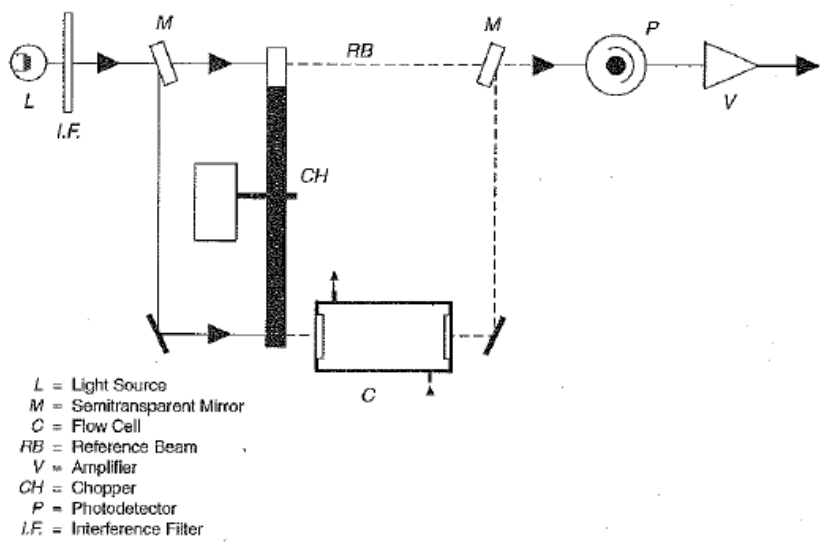

Figure 1.7. Two beams and a single detector spectrometer configuration (AWWA, 2002)

A rotating disk, or "chopper" is inserted to alternatively pass the measurement and reference beams to the same photodetector. As a result, both the luminosity fluctuations of the light source and changes in photodetector sensitivity are eliminated as sources of errors.

Although the most common measurement is done at wavelength 254 nm, scanning through a continuous spectrum will give us more information. The footprint of the signal for the whole spectrum can give us additional information on the compounds present in water. A correlation could be established between the concentration of an organic compound and the measurement signal at selected wavelengths.

Spectro::lyser (s::can, Austria) is a compact and submersible spectrometer capable of online measurements of absorption spectra (from 200 to 750 nm) directly in liquid media in real time. The instrument, shown in Figure 1.8, is a probe of about 0.6 m in length and with a 44 mm diameter. To the best of our knowledge, there only exists one other commercial probe with very similar characteristics: ISIS II (Bran + Luebbe). Spectral information between 200 and 710 nm is used to calculate the concentration of nitrate, suspended solids, organic compounds, DOC, COD, SAC and other paremetres.

A prototype by the Spanish company ADASA has been also identified. Correlation studies between wastewater effluents and UV spectra have been executed in order to perform the calibration of the instrument (Platikanov et al., 2014).

Figure 1.8. Picture of the Spectro::lyser at s::can datasheet (s-can.at) and the one used for this thesis at the Sant Joan Despí DWTP (Barcelona)

The probe consists of three main components: the emitter, measuring cell and receiving unit. A general diagram can be seen in Figure 1.9. The central element of the emitter is a light source, a xenon flash lamp. This is complemented by an optical system to guide the light beam and an electronic control system to operate the lamp. A second light beam within the probe, called a compensation beam, is guided across an internal comparison section, enabling the identification of disturbances in the measuring process. Extinction or absorbance represents a ratio of two light intensities: the intensity of light after the beam has passed through the medium to be measured and the intensity of light determined after the beam has passed through a so-called reference medium (distilled water).

An optical system focuses the measuring and compensation beams at the entrance port of the detector. The light received by the detector is split up into its wavelengths and guided to the 256 fixed photodiodes.

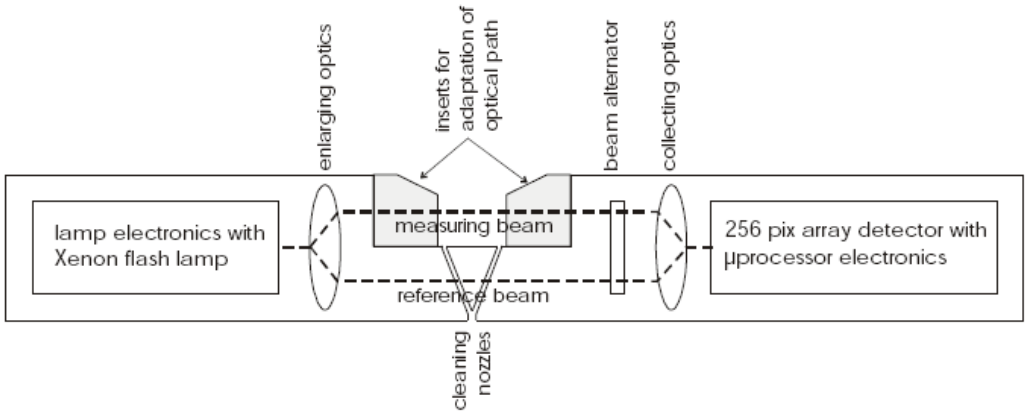

Figure 1.9. Diagram of the functioning of the UV-VIS spectrometer probe (Ziglio 2002)

A major influence on in-situ absorption measurements is turbidity due to suspended substances that cause light scattering, shading and thus influences absorption over the entire spectrum. Turbidity compensation has two tasks: the measurement of turbidity/suspended solid, and baseline compensation for the measurement of dissolved substances. A global calibration is provided as the default configuration of the UV-Vis spectrometer. For many purposes, such as plant control, precision is more important than trueness and the global calibration often delivers sufficient results. Through a second calibration step (local calibration), improvements concerning trueness, precision and long-term stability of the results can be achieved.

The UV-Vis spectrometer provides a global calibration that is based on a Partial-Least-Square (PLS) regression for the paremetres of concern. The broad range of available wavelengths allows high flexibility for the choice of the best correlating wavelengths for the calibration function and the avoidance of cross sensitivities.

Due to the different composition of some types of waters, a local calibration may be required. Local calibration is based on grab samples analysed for the paremetres of interest and can be performed without dismantling the probe. The experience showed that most of the times the laboratory data are the critical part of the calibration procedure. Therefore it is essential to guarantee the quality of the laboratory measurements to obtain good calibration results. The local calibration can be performed by running a complete calibration procedure based on PLS regression (Langergraber et al., 2004). However, in some cases, a change of the slope intercept of the regression function that can be easily done by the user is enough to improve the calibration.

Tests with a spectro::lyser probe have already been performed. Some of the studies include the application of probes for the detection of changes in wastewater quality influent to protect the technologies operating and the detection of industrial waste discharged to a municipal sewer network (Langergraber et al., 2006). For the measurement of ozone, a spectral algorithm was developed that allows quantification in situ using the probe. Furthermore, a spectral algorithm was developed that predicts AOC formation during ozonation and subsequent removal in further treatment steps (van den Broeke et al., 2008). A significant correlation was observed between laboratory analysis and spectro::lyser measurements for chemical oxygen demand (COD) and biological oxygen demand (BOD) concentrations in wastewater discharge in the city of Novi Sad, Serbia (Mihajlović et al., 2014). UV absorbance spectrophotometer installed for water quality monitoring at a conventional drinking water treatment plant was used to develop surrogate paremetres for treatment process monitoring and optimisation (Byrne et al., 2011).

1.5.3. On-line toxicity monitoring systems based on bacteria tests

Biologically based monitoring provides a relative, nonspecific indication that something may be wrong (e.g. a toxic spill has occurred or pathogens have been flush into water supply system) rather than precise, reportable measurement of specific variables (e.g. the concentration of a specific pesticide or pathogen). Biomonitors are used as alert systems to warn that something is wrong, but give little information about what exactly is wrong.

There are limits to the chemicals that can be analysed in water. The magnitude of the total number of possible paremetres, and the inability to monitor them all continuously, has led to the use of biomonitors that can be put online to continuously monitor water quality. Biomonitors involve the use of living organisms (fish, mussels, daphnids, algae, bacteria) and measure the stress placed on the organisms by the presence of toxic materials. Different organisms are tested with the aim of detecting effects of water pollutants on several trophic levels.

Toxicity tests that will be performed in the framework of the research work are based on luminescent bacteria. Tests have been developed based on the reduction on the light emitted by the marine bacterium *Photobacterium phosphoreum*, sometimes referred to as *Vibrio fischeri*. Reference water and sample water are placed in vials that contain a predetermined amount of bacteria. Fluorescence is measured after a certain exposure time (15 or 30 minutes). The decrease in the light emission due to the presence of a toxic substance, either as a ratio or a percentage, is measured. This technique is common in the wastewater industry. An automatisation of this system will be used for research purposes.

Figure 1.10. Picture of the new iTOXcontrol (microLAN flyer) and the TOXcontrol at the Sant Joan Despí DWTP (Barcelona)

TOXcontrol™ (microLAN, Netherlands) is an automatic on-line water toxicity monitor shown in Figure 1.10. It can monitor toxicity in surface water, groundwater, raw wastewater and treated wastewater through the use of luminescence bacteria. It is being proposed for the early warning of water quality in order to ensure the safety of natural waters, by detecting pollution caused by sudden chemical spills and terrorist incidents.The on-line toxicity monitoring TOXcontrol™ structure is divided into three subunits: Bio-monitor, TOXbioshaker™ to cultivate the luminescence bacteria, and TOXview™ used for data treatment and the on-line service.

It uses the luminescence of the bacteria to give an indication of the acute toxicity of the contaminants in water as a function of the emitted light. After the mix of the luminescent bacteria and the water sample, any toxic material in the sample would alter the metabolism of the bacteria. The decrease of light intensity is directly proportional to the concentration of toxic substances in the sample. Before performing the measure, dry frozen bacteria need to be re-hydrated by adding cultivation media and mixing under controlled temperature conditions for several days. Bacteria can react to several families of pollutants that can be found in water, including pesticides, herbicides, PCBs, PAHs, heavy metals, petroleum-related pollutants, protease inhibitors, respiratory system inhibitors and so on.

The equipment works on the same basis as the certified methodology for the analysis of toxicity using *Vibrio fischeri*(ISO 11348-3) but adapted to automatic equipment. The analyser works in two parallel lines. While one of the lines is preparing the mixture of the bacteria solution with sample water and measuring the effect of that sample on bacteria, a second line is using reference water instead of sample water, as the output data is a relative measurement of the light emitted by the first line compared to the

second one. The bacteria solution is prepared using a (2% NaCl) solution. Figure 1.11 shows the bio-monitor analyser.

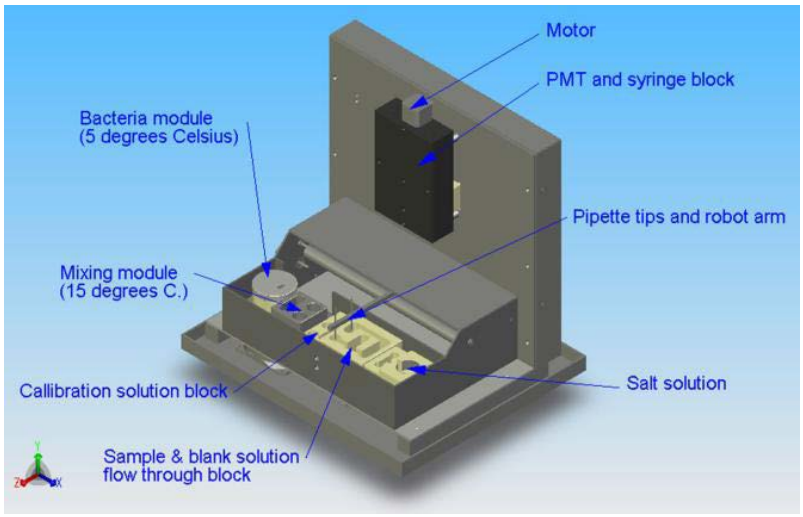

Figure 1.11. Diagram of the biomonitor analyser

TOXcontrol™ was chosen as a case study in the European project TESTNET (Towards European Sectorial Testing Networks for Environmentally sound Technologies) with the aim of adapting existing verification protocols developed for chemical monitors to be able to handle bio-monitors (Appels et al., 2007). The instrument has also been identified as a rapid instrument for real-time measurements (Storey et al., 2011).

1.6. Water quality indicators

Monitoring actions should be based not only on the quantification of physical-chemical, chemical and biological paremetres. An assessment of the potential of these pollutants to have a chemical toxic effect on ecosystems or human health is the most important process in order to establish preventive or corrective actions.

The risk assessment process, in relation both to human health and the environment, includes a sequence of actions (Joint Research Centre, 2003):

- Assessment of effects, comprising
    - Hazard identification: identification of the adverse effects which a substance has on inherent capacity to cause; and
    - Dose-response assessment: estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect, where appropriate.

- Exposure assessment: estimation of the concentrations/doses to which human populations or ecosystem receptors are or may be exposed.
- Risk characterisation: estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include "risk estimation", i.e. the quantification of that likelihood.

1.6.1. Indexes for measuring the impact of pollutants on aquatic ecosystems

A contaminant present at low concentration levels for a long exposure time can have different effects on ecosystems depending on its toxicity. The paradigm of toxicity relies on a relationship between the toxic concentration and the fraction of the population affected by an aquatic ecosystem receptor and by a group of aquatic receptors (algae, crustaceans and fishes). The relationship for chronic exposures and for ecosystems used to be defined as*Predicted Non-Effect Concentration* (PNEC), that is, a concentration in water or food that affects less than the 5% of the ecosystem.

A risk evaluation of toxicity caused by pollutants in aquatic ecosystems is a key methodology to assess the status of a river water body and can be a useful tool for regulators when they have to establish threshold values to control contamination. As an example, the relationship between ecological status and chemical status can be considered as one of the key issues of the WFD (Ginebreda et al., 2010).

Stressors related to the impact that is observed on aquatic ecosystems (e.g. density and diversity of macroinvertebrates) are diverse and can include chemicals and physical-chemical agents (temperature, lack of oxygen, pH, salinity, suspended solids) as well as habitat related paremetres (latitude, length, drainage area), effluent characteristics and other paremetres that are difficult to measure. If scope is focused on toxicity, only a small part (often less than 10%) can be assigned to known substances. This means that even if there is a relationship between chemicals and toxic effects, known analysed chemicals do not always give the ecological status of an aquatic ecosystem, and therefore correlation between observed ecological effects and known contaminants would not be straightforward (Guillén et al., 2012).

Monitoring measurements at river basin scale need to be based on a list of candidates, but they must be integrated into a methodology capable of analysing evidence and characterising the causes to finally identify, if possible, the stressors and the linked sources of impact. The proposed indicators will be focused on the integration of several paremetres measured by classical analytical chemical methods in order to assess the impact on aquatic ecosystems. Some other indicators have been previously developed and they have been used to assess the impact due to eutrophication or a combination of lack of oxygen and other stressors apart from toxic effects. For example, some local indexes have been already developed

for certain areas, e.g. river basins in Catalonia where the concentration of nitrate, ammonium, phosphates, TOC, conductivity and chlorides are used to give the global level of the physicochemical quality of rivers.(Agència Catalana de l'aigua, 2010).

Local indicators for river aquatic ecosystems assessment should be developed based on the pollution characteristic of an area in order to specifically screen potential chemical stressors. Risk assessment should be tailored for local conditions, so compound prioritisation would depend on the particularities of each river basin (Ginebreda et al., 2014). These indicators can be calculated using experimental and public data. Matrices implemented in mathematical programs can help in the processing of data to create these indicators.

The environmental risk assessment approach attempts to address the concern about the potential impact of substances on the environment by examining exposures resulting from releases of chemicals and the effects of such emissions on the structure and function of the ecosystem. For this examination, quantitative PEC/PNEC estimation for the environmental risk assessment of a substance comparing Predicted Environmental Concentrations (PEC) with Predicted No Effect Concentration (PNEC), that is, the concentration below which unacceptable effects on organisms will most likely not occur (Joint Research Centre, 2003).

In this thesis, historical data on the occurrence of the substances in the Llobregat River will be used as PEC. Although concentration measurements may have a significant uncertainty due to the temporal and spatial variation of the presence of the compounds, it has been assumed that the reliability of results will be greater than by using predicted data based on models. Llobregat River has been the basis of several risk assessment studies in recent years by the development of new indexes in order to help to identify the compounds that should be addressed by the Public Administration in its policies (Fàbrega et al., 2013; Kuzmanović et al., 2015).

PNEC should be calculated for the aquatic environment, thereby ensuring an overall protection of the environment. An extrapolation is made from single-species toxicity data assuming that the ecosystem sensitivity depends on the most sensitive species, and the protection of the ecosystem structure protects community function. For most substances, the number of studies needed to find out their toxicity has not been achieved yet, so the pool of data that is available to predict ecosystem effects is very limited. In this case, the assessment factors must be used. The size of the assessment factor depends on the confidence with which a $PNEC_{water}$ can be derived from the available data, as can be seen in Table 1.6.

Table 1.6. Assessment factors to derive an aquatic PNEC (Joint Research Centre, 2003)

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, Daphnia and algae)	1000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case)
Field data or model ecosystems	Reviewed on a case-by-case basis

When assessing the status of the ecosystem, it is also important to consider the toxic effects in the higher members of the food chain, either living in the aquatic or terrestrial environment, which result from the ingestion of organisms from lower trophic levels that contain accumulated substances. Bioconcentration and bioaccumulation may be of concern for lipophilic organic chemicals and some metal compounds as both direct and indirect toxic effects may be observed in long-term exposure.

One way to assess the risk of bioaccumulation in aquatic species is to measure the Bioconcentration Factor (BCF), that is, the ratio between the concentration in the organism and the concentration in water in an equilibrium situation. The potential for bioaccumulation can be estimated from the value of the octanol-water partition coefficient (log Kow).The BCF approach makes it possible to estimate the chemical risk for terrestrial vertebrates by using PNEC values expressed in a food basis.The PEC values expressed in food (worms, plants) can be obtained from environmental compartments such as water.

1.6.2. Indexes for measuring the impact of pollutants on human health

According to the World Health Organisation (WHO), the quality of water, whether used for drinking, domestic purposes, food production or recreational purposes has an important impact on health. Water of poor quality can cause disease outbreaks and it can contribute to background rates of disease manifesting themselves on different time scales (WHO, 2013).

It is widely accepted that all stakeholders responsible for water safety should take efforts to improve risk management and risk communication to consumers, that is, the provision of information and health-based assessments on the various microbial, chemical, radiological and physical human health hazards that may be present in the water cycle. Health-based assessments of existing and emerging hazards in water should include a proper monitoring of the water at source and the produced water, technologies for treating water designed for reducing risks and an adequate approach to managing those associated risks.

The European Union ensures that drinking water quality is controlled through standards based on the latest scientific evidence. The Drinking Water Directive (98/83/EC) concerns the quality of water intended for human consumption. A total of 48 microbiological, chemical and indicator paremetres must be monitored and tested regularly. In general, the WHO's guidelines for drinking water and the opinion of the Commission's Scientific Advisory Committee are used as the scientific basis for the quality standards in the drinking water (WHO, 2008).

Water safety plans are considered by the WHO as the most effective means of maintaining a safe supply of drinking water to the public. Hazards and risks need to be identified, and appropriate steps towards minimising these risks are then investigated (WHO, 2005).

In developed countries, a wide implementation of water treating technologies and proper management has led to a significant reduction in the risks associated with water ingestion. Good practices have led to a decrease of the pollution at source and to a better removal of contaminants in water. Nevertheless, the list of contaminants that should be taken into account is growing as the studies to define the effects on health are progressing.

Assessing exposure and the health consequences of chemicals in drinking water is challenging. Exposures are typically at low concentrations, measurements in water are frequently insufficient, chemicals are present in mixtures, exposure periods are usually long, multiple exposure routes may be involved, and valid biomarkers reflecting the relevant exposure period are scarce. In addition, the magnitude of the relative risks tends to be small (Villanueva et al., 2013).

Indexes need to be established not only in relation to affected ecosystems but also those related to an impact on human health. As contamination reaches water bodies, effects on human health should be avoided when this water is taken and treated for drinking purposes. It is of utmost importance to assess what level of efficiency water treatment technologies should achieve in order to reach a level that is acceptable for human consumption.

Not only the removal of incoming pollutants need to be assessed. Other products that could be also harmful, are produced as a result of treatment technologies. Disinfection processes are the cause of the formation of Disinfection By-Products (DBPs), especially when the precursors are present and the optimum conditions (temperature, etc.) exist. According to the World Health Organisation (WHO, 2011), trihalomethanes (THMs) are the most abundant DBPs, and the United States Environmental Protection Agency (USEPA) has stated that these are human carcinogens.

A methodology approach is based on toxicity assessment, exposure assessment, risk indexes and comparison with the target risk that must be observed for every substance. Exposure assessment included in this thesis is focused on water ingestion and is based on the dose of contaminant ingested through this pathway.

Among the databases that offer information on the toxicity of the compounds that can be found in water for toxicity assessment, two of the most widely used are the Risk Assessment Information System (RAIS) and the WHO guidelines (WHO 2011). Legislation can also act a source for reference data on the potential effect of compounds, as the thresholds established are a consequence of toxicity studies.

The manner in which the risk is expressed depends on the nature of the hazard and the types of data upon which the assessment is based. Risk estimates for adverse effects other than cancer are usually expressed as the ratio of the toxicological potency of the chemical to the estimated dose or exposure level received. That is the case with systemic toxicity, which refers to adverse effects on any organ system following absorption and distribution of a chemical throughout the body. The systemic indexes bellow unit expresses no effect and therefore an acceptable risk.

In this case, the Reference Dose (RfD) is an estimate of the lowest daily human exposure that is likely to occur without an appreciable risk of deleterious effects during a lifetime. Deriving an RfD involves determining a No Observed Adverse Effect Level (NOAEL) or the Lowest Observed Adverse Effect Level (LOAEL) from an appropriate toxicological or epidemiological study, and then applying uncertainty factors to arrive at the RfD. The NOAEL is the highest exposure level that can occur without statistically or biologically significant adverse effects, and the LOAEL is the lowest exposure level at which adverse effects have been shown to occur.

Carcinogenic effects are tumours caused by cancer. A key distinction between cancer and other toxicological effects is that most carcinogens are assumed to have no dose threshold. Cancer risks are most often expressed as the probability of an individual developing cancer over a lifetime of exposure to the chemical in question. This means that this index is a kind of quantitation of the probability of developing tumours. Generally a target carcinogenic acceptable risk value of 10^{-5} or 10^{-6} is used.

1.7. Objectives of the research work

1.7.1. Motivation and purpose of the thesis

The main objective of the thesis is the development and optimisation of methodologies for the chemical and biological characterisation of water quality at source (surface waters) and produced water (drinking water for consumption) with the final aim of integrating emerging technologies into the monitoring processes.

The thesis has been developed on a specific case study: the city of Barcelona (NE Spain) and its main source for drinking water, the Llobregat River. The river has a low average flow and is highly impacted by human activities. Public Administration must safeguard quality of the river waters in order to attain a good ecological status. Water operators are facing a big challenge everyday in treating these natural waters in order to make them suitable for human consumption. The quality of final waters will depend mainly on the characteristics of the source water and the removal efficiency of treating technologies.

It is important to propose new methodologies that address the needs of these final users. Requirements concerning the cost of the monitoring techniques and the information they provide are key in order to make the final selection. In most cases, the best solution is a combination of technologies. Some may provide accurate information on the concentration of target pollutants while others are intended to give a rapid alarm if an alteration to the global quality of water occurs. Additionally, working with data in order to obtain indexes based on the potential harm that a substance can pose to the ecosystem or the public may be possible with more useful information than just the occurrence of a given pollutant. The scientific community agrees that risk assessment need to be included as part of water quality legislation.

In the framework of this thesis, a combination of techniques has been tested and are detailed below as specific objectives:

- Optimisation of methodologies based on off-line techniques in the laboratory including solid phase extraction, liquid chromatography and mass spectrometry for the identification and quantification of a selection of emerging pollutants (pharmaceutical compounds)
- Integration and validation of emerging biosensing technologies for on-line automatic measurement of global toxicity of surface waters by inhibition of *Vibrio fischeri* luminescence
- Development of methods based on an in-line UV-Vis spectrophotometer for real-time monitoring of physical-chemical paremetres in rivers (early warning system) and drinking water (prediction of blends of different sources)

- Proposal of indexes for measuring the ecological impact of contaminants on aquatic and terrestrial ecosystems
- Creation of indexes for evaluating the efficiency of water treatment technologies and assessing the potential impact of contaminants in drinking water to the supplied population

The methodology for achieving each of the specific objectives has been described in individual chapters. Each of the tasks is based on different technologies or methods, although all of them are focused on achieving a single overall objective.

The aim of this thesis is to help to implement technologies and risk analysis methodologies that would help to gain a better understanding of pollutants occurrence and behaviour in water. In addition to this, the proposed devices have the potential to become the basic building blocks for intelligent monitoring of water quality paremetres. Such sensor devices networks would assist the implementation of the WFD and related directives by providing data on pollutant levels in surface and drinking waters at higher temporal frequencies than is currently feasible using current monitoring regimes (typically based on spot sampling followed by laboratory analysis). New instruments should provide information that is useful for water operators and the public administration in decision-making processes.

1.7.2. Scope and organisation of the thesis

The thesis was prepared mainly within the context of two national research projects; the VIECO project (009/RN08/01.1) dealing with advanced strategies for the monitoring of surface waters and the WATMATIN project (CTM2010-21182) based on the quality control of drinking water at the exit of the DWTP and the distribution network. The global diagram can be seen in Figure 1.12.

In order to identify which pollutants are commonly found in the Llobregat River, and to acquire knowledge about their toxicity, we carried out bibliographic research and consultation of public and private data bases. Monitoring plans of surface waters at the intake of the DWTP are performed in a more comprehensive way by water operators than the public administration because of their impact on drinking waters. Nevertheless, both databases are taken into account. Concerning emerging pollutants, they are not monitored on a routine basis, so scientific publications have been reviewed in order to find information about their occurrence and toxicity on ecosystems (Chapter 2). Most of the monitoring campaigns for these emerging compounds have been executed as part of research projects.

In order to select the best instrument that meets the needs of the final users, a technological review and benchmarking of available instruments needs to be carried out. The selection of the on-site instruments

included in this thesis was made after a benchmark of commercial devices. Additionally, the selection of an instrument for the identification and quantification of *Escherichia coli* was made within the framework of a privately funded project. A complete review of the technologies, prototypes and commercial devices was completed in order to assess which technology best fits the users' requirements. Not only analytical performance but the financial cost should be taken into account. A commercial instrument based on enzymatic fluorescence reaction was selected and tested in a real case scenario. The review of technologies was published and has been included as Chapter 3. Unfortunately, the results of the validation test have been excluded due to the confidentiality of the project.

Beyond legislation, there are a large number of compounds whose effects on the health of ecosystems or the human population is not well established. For this reason, those families of substances have not been included in the legislation. However, Decision 2015/495 of 20 March 2015 specifically mentions pharmacueticals that have been included in the watch list of substances for monitoring: estradiol, estrone, ethynylestradiol, diclofenac, erythromycin, clarithromycin and azithromycin. For the unequivocal identification and quantification of individual pharmaceuticals at the levels that are found in the environment (in the order of ngL^{-1}), the only alternative nowadays is the use of advanced instruments in the laboratory. Chapter 4 includes the optimisation of a methodology based on liquid chromatography coupled to mass spectrometry (triple quadrupole and time-of-flight) aimed at reporting results of concentrations found in several campaigns in the Llobregat River.

The VIECO project proposed a platform for the water quality monitoring at the intake of the Barcelona DWTP (Chapter 5). The platform integrated different strategies including a combination of two instruments able to act as an Early Warning System, a UV-Vis probe for measuring alterations in the physical chemical characteristics of the water and a monitor to give indications of the global toxicity of the water. The platform was tested over several months at the Llobregat River. Both systems had been designed and validated in rivers from central Europe, having very different characteristics from the Llobregat River. The techniques faced the challenge of being tested in a river with high fluctuations, not only in flow, but also in some quality paremetres such as turbidity. Instruments are usually calibrated for a narrow range of target paremetres, but the response in fluctuating environments is not always guaranteed.

The biomonitor used in the proposed platform described in Chapter 5 is based on the inhibition of fluorescence of the bacteria *V. fischeri*. The technique is based on the same principle as the commonly used laboratory instrument Microtox™. The main difference is the automation of the methodology. What can be seen as an improvement entails some disadvantages. The certified methodology, as well as most of the toxicity studies measuring the inhibition in front of specific contaminants, is based on Microtox™. In Chapter 6, an evaluation of the response of the on-line biomonitor was performed for a selection of

priority pollutants commonly found in the Llobregat River waters, comparing the results with two standardised techniques: Microtox™ and *Daphnia magna* tests.

Source water has a strong impact on the characteristics of drinking water. Organic matter, for example, although removed to a large degree in the DWTP, remains in a small quantity that is enough to leave a fingerprint in drinking water when analysed by UV-Vis spectrophotometry. In a case like Barcelona, where up to 5 different sources for drinking water can exist (3 DWTPs located in 2 different rivers, seawater, groundwater), the water that can be found in the distribution network is a blend from different sources. For water operators, it is important to find out which is the approximate percentage of every origin contributing to the blending. Nowadays, the information is obtained by combining hydraulic models and conductivity measures. A more advanced method is proposed in Chapter 7 by analysing the fingerprint of the water sample and comparing it with the fingerprint of the water produced at every origin through chemometric methods.

In order to report the ecological status of a water body, the WFD requires the development of indicators. An effort was made to create biological indicators based on the presence of local species in order to express the ecological status of surface waters. From a chemical point of view, a list of pollutants (45 according to the recently updated legislation) should be analysed and their peak concentrations and annual averages reported. The financial investment required to analyse 45 chemical paremetres is significant and nowadays there are public administrations that cannot afford this. A new strategy based on risk assessment and management should be promoted. For this purpose, risk indexes have been developed in Chapter 8 as a complementary methodology based on the local occurrence of a large list of compounds. The global indexes have been created on the basis of historical data and every pollutant has a different weight depending on its toxicological effect on aquatic and terrestrial organisms. These indexes should serve to support public administrations in the decision-making process.

As has been mentioned previously, risk assessment methodologies are being proposed as an alternative to report concentrations of a definite list of compounds. For drinking water, the hazard of the substances existing in water may be reported as indexes, comparing their concentration with a reference value. The reference value can be obtained from public databases where the value that has been calculated from toxicological studies is shown. Chapter 9 presents the global indexes that have been developed in the Sant Joan Despí DWTP in Barcelona in order to analyse the temporal variation of the toxicological impact of this drinking water, and to assess the variation of the index due to the upgrade of the technologies operating in the DWTP.

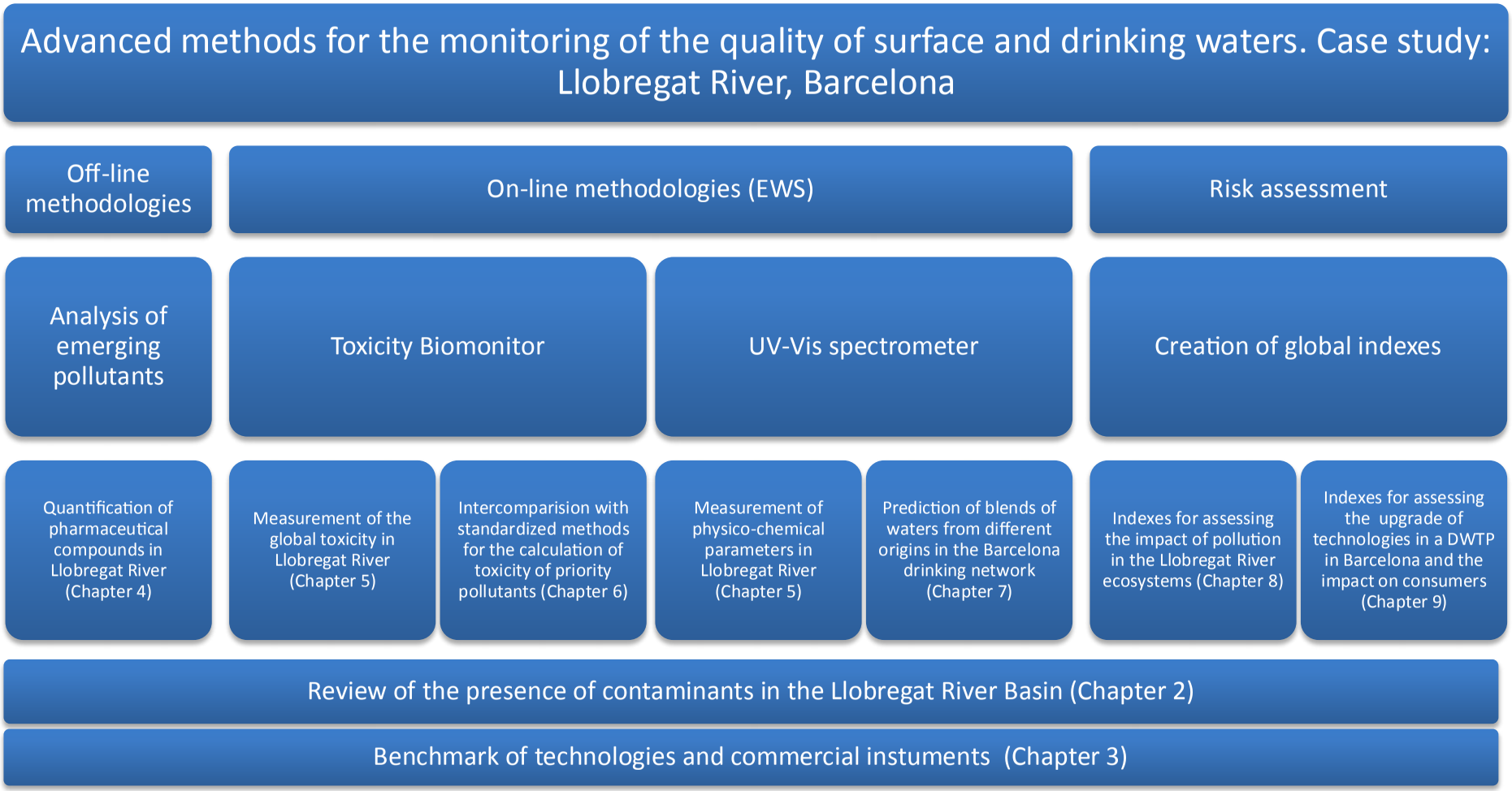

Figure 1.12. Diagram showing the organisation of the thesis

1.8. References

76/464/EEC Council Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community.

91/271/EEC Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment.

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Presence and biological effects of emerging contaminants in the Llobregat River Basin: a review

2. Presence and biological effects of emerging contaminants in the Llobregat River Basin: a review

2.1. Abstract

The Llobregat River (north-east Spain) is the largest drinking water source for Barcelona and its surrounding area. As one of the only water sources in the area, the river waters have been overexploited and effluents from more than 30 urban wastewater treatment plants, industries and agriculture runoffs have been discharged into the river. This study reviews the presence of emerging contaminants published in recent decades, emphasising the observed effects on ecosystems caused by the contamination. Pesticides, surfactants, oestrogens, pharmaceuticals and personal care products and even illegal drugs are the main groups detected in different studies, reporting alterations in species composition, the abundance of biomass and endocrine disruption measured by alterations in enzymatic activity or specific protein production.

There is evidence that the information available provides an overview of the river status but not a representative picture according to the Water Framework Directive.

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2.2. Introduction

The European Water Framework Directive (WFD) (Directive 2000/60/EC) establishes bases to regulate water resources with the aim of conserving, protecting and improving their quality and sustainable use. The WFD requires that all surface waters must reach a good chemical and ecological status in a qualitative and in a quantitative sense by 2015. For WFD fulfilment it is necessary to know the status of water resources at the moment in order to establish which technologies should be implemented to achieve the goals of the WFD.

Several studies have focused on the presence of organic contaminants in the Llobregat River, the largest drinking water source for Barcelona and its surrounding area, where a broad range of contaminants have been detected. Inorganics also have been studied but most of the studies focus only on sediment accumulation, especially metal ions and non-metals such as arsenic. Most of these studies have been complemented by ecological status monitoring studies, mainly based on the effects of endocrine disrupting compounds.

This study reviews the most significant monitoring programmes that have been launched within the Llobregat River and its tributaries in order to discover its water quality and the results obtained by several research studies dealing with the evaluation of the effects of this contamination. It also discusses the use of monitoring programmes to provide valuable information on the biological health of the ecosystems.

To our knowledge, this work represents the first review that is solely focused on the Llobregat River.

2.3. The Llobregat River

The Llobregat River emerges in Castellar de n'Hug, in the north west of Catalonia (Spain), at an altitude of 1400m and flows approximately 160 km before reaching the Mediterranean Sea, 10 km south of Barcelona (see Figure 2.1).

The Cardener and Anoia Rivers are the main tributaries. In the lower-middle course of the Llobregat and Cardener Rivers there is a large concentration of industries, agricultural activities and densely populated areas with major water demands. In contrast, the Anoia River is mainly influenced by agricultural area (vineyards) and industries. As a Mediterranean river, it is highly dependent on climatic conditions (see Figure 2.2A) and the flow can range from several hundred m3/s in the storm period, normally in spring

and autumn to low m3/s during summer (dry period) where the flow can decrease considerably leading to worse water quality due to the increase of effluent wastewaters in the total flow of the river and when the dilution factor can be negligible.

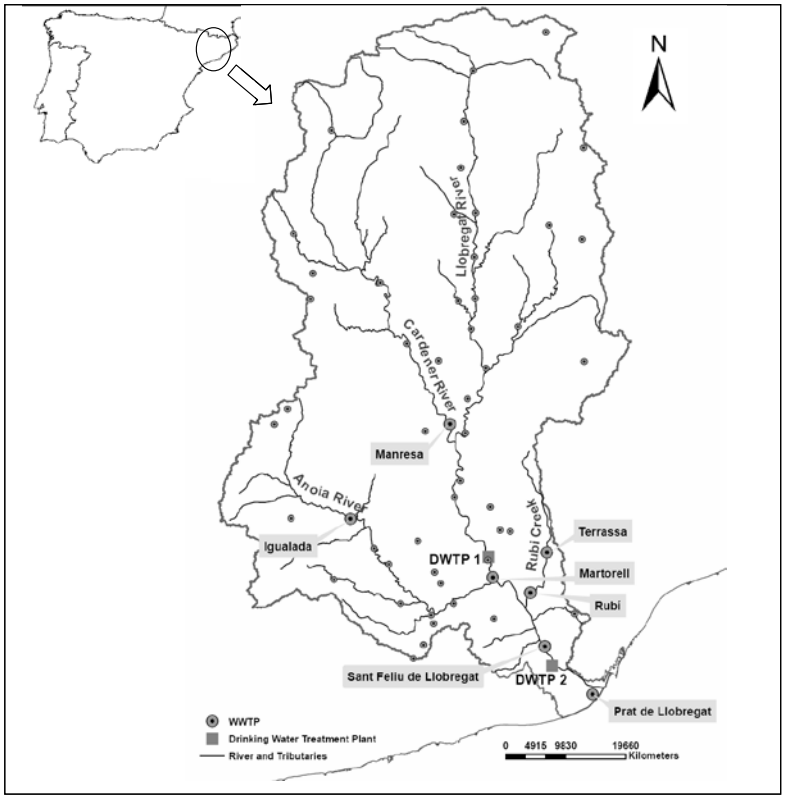

Figure 2.1. Geographical location and map of the Llobregat River basin. DWTP1: Abrera, DWTP2: Sant Joan Despí. WWTP with more than 100,000 population equivalents are named

This basin is also characterised by its salinity from salt mines located in the upper course of the river. Although in the nineties a collector was built with the aim of collecting the mining lixiviates, the salinity problems in the Llobregat basin were not totally solved and high salinity levels are still found at some points (see Figure 2.2 B).

To improve the water quality of the Llobregat River and its tributaries, more than 30 WWTPs treating a mixture of urban or industrial wastewaters have been set up along the river, as shown in Figure 2.1. The main industries sited along the Llobregat River are tannery, food products, textile, pulp and paper industries discharging a broad spectrum of organic chemicals into the river. Therefore, the river receives effluents from these WWTPs and surface runoff from agricultural areas.

The removal of contaminants by WWTPs is in some cases not complete; consequently they can enter into the environment via sewage effluents and thus become a potential risk to the receiving bodies and in addition, into the production of drinking water.

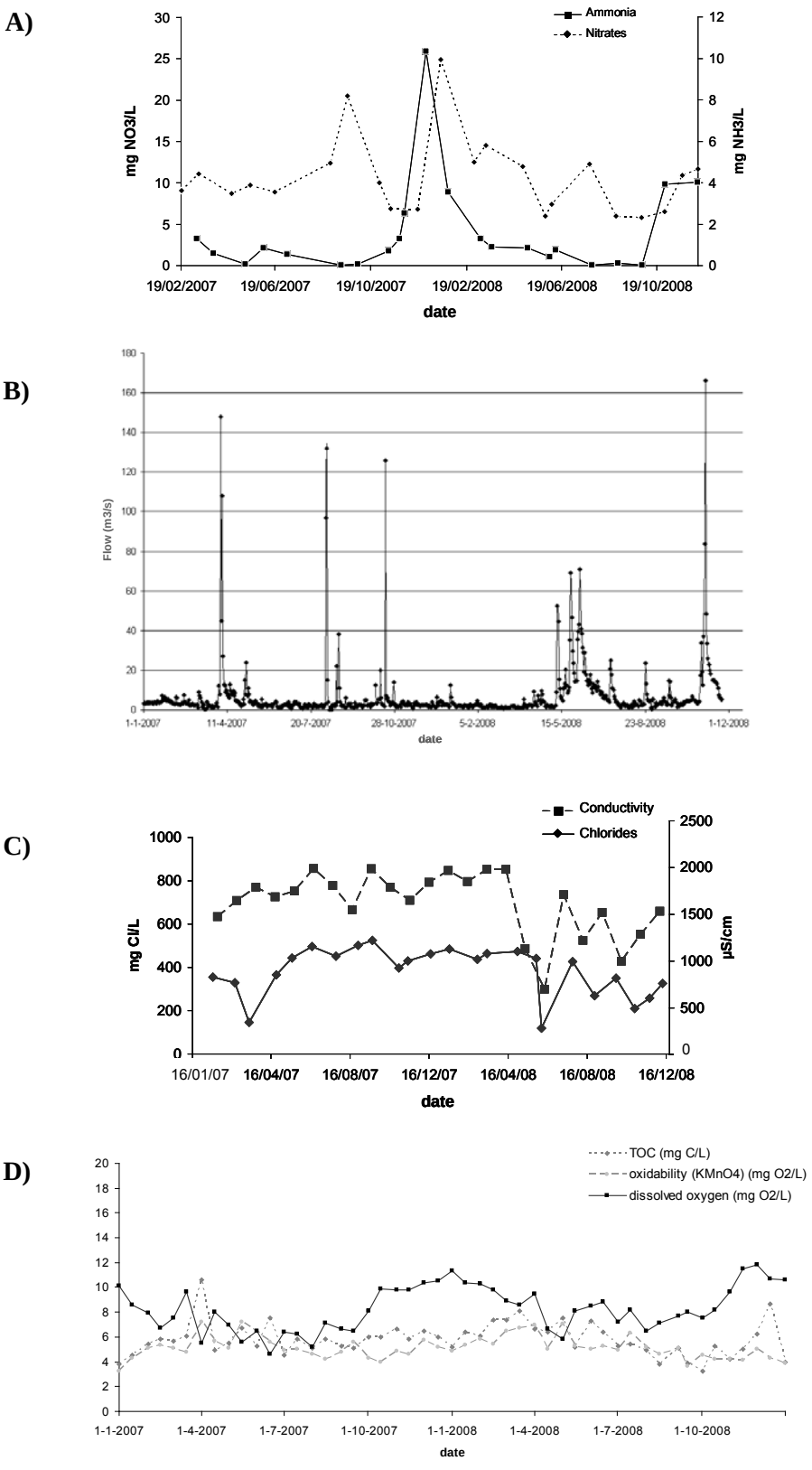

Figure 2.2. Data paremetres of the Llobregat River in the low course during 2007/2008. A) flow B) conductivity/chlorides C) total organic carbon (TOC)/oxidability/dissolved oxygen and D) ammonia/nitrates

The Llobregat River is one of the main drinking water sources in the area due to the scarcity of groundwater resources. Therefore, the quality of the raw water must be controlled and to do this a net of surface water quality control stations have been set up in recent decades providing data on general paremetres such as the ones depicted in Figure 2.2 where a summary of general paremetres in the low course of the Llobregat River is presented. To enhance the water quality of the river, several by-passes have been constructed along the river avoiding the most contaminated parts of the river to reach the drinking water treatment plants (DWTP). Abrera DWTP and Sant Joan Despí DWTP, located in the lower-middle course of the Llobregat River (see Figure 2.1), supply approximately 40% of drinking water to the Barcelona Metropolitan and surrounding area, whose characteristics are shown in Table 2.1.

Table 2.1. Characteristics of the two main DWTP operating in the Llobregat River

DWTP	% of population served in Barcelona area	Treatment	Maximum water production	Source	Treated water
Abrera (DWTP 1)[1]	5%	Screening, sandfree, chlorination, flocculation and settling, sand filtration, filtration through activated carbon, electrodialysis-reversal, final chlorination	4 m^3/s	97% river water-3% groundwater	52.9 Hm^3/year
Sant Joan Despí (DWTP 2)[2]	37%	Predioxichlorination, grit removal, coagulation-sedimentation, sand filtration, ozonisation, filtration through activated carbon, ultrafiltration, reverse osmosis, hardness adjustment, final chlorination	5.3 m^3/s	75% river water-25% groundwater	107 Hm^3/year

Source: [1]. ATLL, Annual report, 2008; [2]. Internal source, 2009

2.4. Levels of contaminants in Llobregat River waters

The Llobregat River has been the subject of several studies dealing with the presence of contaminants in surface water and related compartments (e.g. sediments, fish). In this study, only compounds detected in surface waters are taken into account. Most of these studies focus on the lower and middle part of the river basin, where most of the WWTPs, DWTPs and population are located, and which therefore is the area with higher pressures.

2.4.1. Pesticides

Pesticides are a group of compounds widely used in agricultural areas, related industries and other applications that comprise compounds with different physico-chemical characteristics. After their field application they can transfer to water (either surface or ground water) where, depending on the compound solubility, bioaccumulation in living organisms or accumulation in sediments or soils can occur.

The majority of the studies focused on ground water and surface water, due to their importance as a source of drinking water, where EU legislation establishes a maximum acceptable level of 0.1 µg/L for individual compounds in water intended for human consumption (European Union Council Directives 80/778/EEC, 91/414/EEC, 98/83/EC). Moreover, the WFD established a list of 33 priority substances to be controlled in the field of water policy (Directive 2008/105/EC), of which a third are pesticides.

Due to the importance of these compounds, their presence has been extensively studied in the Llobregat River and more specifically in the intake of both DWTPs located in the river in order to discover the quality of the intake water (Kampioti et al., 2005; Quintana et al., 2001; Rodriguez-Mozaz et al., 2004). In those studies, pesticides belonging to different classes (triazines, phenylureas, organophosphates, anilines, acidic, thiocarbamate) have been analysed where MCPA (acidic), dimethoate (organophosphate), diuron (phenylurea), terbuthylazine, simazine and atrazine (triazines) were the compounds more frequent detected and in higher concentrations (up to 2.21 µg/L for simazine, 463 ng/L for atrazine, 415 ng/L for MCPA and 239 ng/L for diuron). However, the concentrations detected varied depending on the season; the higher values were found during the field application period (February-June).

In recent studies (Kuster et al., 2008; Ricart et al., 2010; Terrado et al., 2009) performed at different locations along the Llobregat River and its tributaries, the most ubiquitous and abundant compounds detected were similar to the ones detected in the DWTP intake: the acidic pesticides MCPA (up to 1.28 µg/L) and 2,4-D (up to 109 ng/L); the phenylureas linuron (up to 327 ng/L) and diuron (up to 99.7 ng/L) and the organophosphate diazinon (up to 785 ng/L).

Higher concentrations were detected in previous studies for terbutylazine (13 µg/L) due to a local spill produced by a company that uses it for its refrigerating circuit (Lacorte et al., 1998) and for glyphosate just before field application finding quite high concentrations (20-60 µg/L) with peaks of 137 µg/L after three days. However, after 12 days of field application this was not found any more (Puertolas et al., 2010).

As a summary, all the studies carried out in the river confirm the presence of pesticides in the Llobregat River coming from their usage in the agriculture and industrial activity. The higher concentrations were always found during their application period (spring-summer). However, as can be seen in Figure 2.3 where a summary of pesticide concentration is shown, the concentrations found rarely exceed the EU maximum acceptable concentrations.

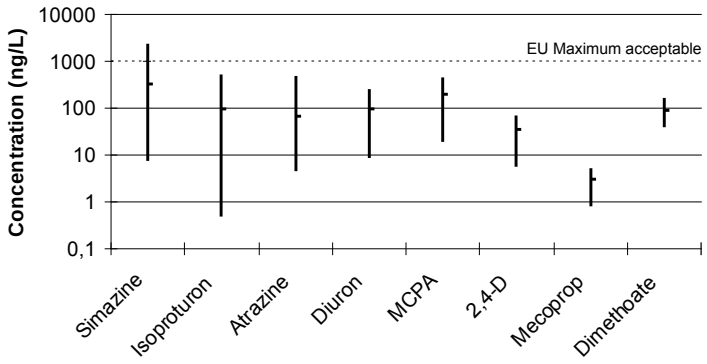

Figure 2.3. Levels of selected pesticides found in the intake of DWTP 2, showed as maximum, minimum and average concentrations. The dotted line shows the maximum acceptable concentration for individual compounds according to EU legislation. (Kampioti et al., 2005; Kuster et al., 2008; Quintana et al., 2001; Rodriguez-Mozaz et al., 2004)

2.4.2. Pharmaceuticals, personal care products and illegal drugs

Interest in the presence of pharmaceuticals, personal care products (PPCPs) and illegal drugs in the environment has increased in recent decades. Their usage is greater than before, mainly due to the increase of density and age of the population, and hundreds of tonnes of these compounds are consumed every year.

After they are excreted, via urinary or faecal excretion, or directly disposed into the sewerage system or discharged through pharmaceutical manufacturing, they can enter surface water, groundwater or even in drinking water mainly through insufficiently treated wastewater effluents (50-90%) (Radjenovic et al., 2007).

A large group of PPCPs have been extensively studied along the Llobregat River during the last decade (Farre et al., 2001; Garcia-Galan et al., 2010; Kuster et al., 2008; Lopez-Roldan et al., 2010; Muñoz et al., 2009). Non-steroidal anti-inflammatories (NSAIDs): ibuprofen and diclofenac; lipid regulators; gemfibrozil and bezafibrate; antibiotics: sulfamethoxazole and ofloxacin and β-blocker metoprolol were the compounds found in higher concentrations. For all the studies, the contamination load increased

downstream along the river, and the highest concentrations were found in the Rubi creek and a channel receiving by passes from the most contaminated parts of the river.

Most of the samples analysed were in the range of 10-1000 ng/L, where the higher concentrations relate to the aforementioned hot spots. Even concentrations exceeding 10 µg/L have been detected for diclofenac, bezafribrate and sulfamethoxazole (Muñoz et al., 2009). However, the concentrations vary greatly depending on the sampling time. As an example, sulfamethoxazole was studied at the same points in two sampling campaigns during June 2005, November 2005, May 2006 for the first sampling campaign (Muñoz et al., 2009) and November 2005, May 2006, November 2006 for the second one (Garcia-Galan et al., 2010). While in the first sampling campaign 11.92 µg/L maximum concentrations and 1.11 µg/L mean concentrations were detected, in the second one these were less than half (4.29 µg/L and 0.25 µg/L, respectively).

Moreover, two of the last published studies (Lopez-Roldan et al., 2010; Muñoz et al., 2009), that focused on a high number of pharmaceuticals (28, belonging to 8 different therapeutic groups), are compared in Figure 2.4. As can be seen, the concentrations can change greatly in a short period of time (a few months). Despite studying the same compounds and in the middle lower part of the river, the first study shows much higher concentrations than the second for all the therapeutic classes except for two. This can only be explained by the fact that the first study covers samples taken in June (summer period), with a low river flow and therefore a pollutant concentration and the second in the autumn-winter period with a higher river flow.

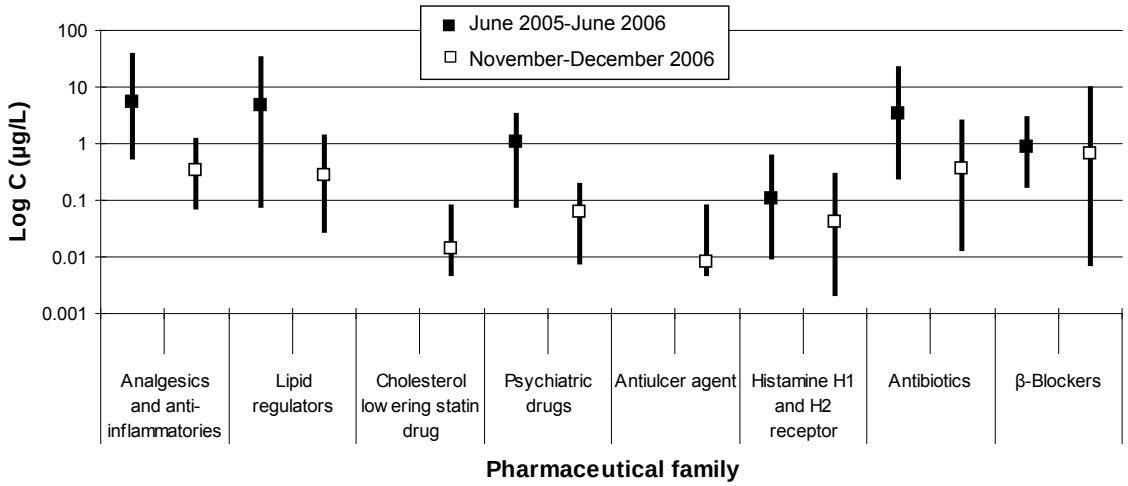

Figure 2.4. Cumulative levels of pharmaceuticals, grouped by therapeutic class detected in two different studies at different sites of the Llobregat. Showed as maximum, minimum and average concentrations. (Lopez-Roldan et al., 2010; Muñoz et al., 2009)

The presence of illegal drugs, a group of compounds causing great concern in recent years, has been also studied in the Llobregat River. Water samples from different points of the Llobregat River (including the intake of DWTP), Cardener and Anoia River and the Rubí creek were collected and analysed to detect the presence of illegal drugs, namely codeine, morphine, THC-COOH (11-nor-9-carboxy-Δ9-tetrahydrocannabinol; the main urinary metabolite of the active component of cannabis), methadone and its degradation product EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) (Boleda et al., 2007; Boleda et al., 2009) and cocaine, its metabolite benzoylecgonine (BE), amphetamine-type-stimulants (ATS), ketamine, phencyclidine (PCP), lysergic acid (LSD) and fentanyl along the river. Stimulatory non-controlled drugs such as nicotine and caffeine were also included due to their high widespread consumption (Huerta-Fontela et al., 2007; Huerta-Fontela et al., 2008). Concentrations tend to be higher along the course to the mouth, due to the increasing population living near the coast. However, higher concentrations of almost all the compounds were found in the Anoia River and in the Rubí creek. The higher concentrations and the higher ubiquity were found as expected for nicotine and caffeine and its degradation product paraxanthine (up to 2.8 µg/L). Illegal drugs were found in ng/L concentration. Codeine, EDDP, THC-COOH and BE were the drugs detected in higher concentrations.

When daily and seasonal variation was studied, relative constant concentrations for nicotine and caffeine and their metabolites were found. For illegal drugs the highest values detected were observed during weekend and were seasonal during summer and winter.

As has been shown, pharmaceuticals are a group of compounds present in Llobregat River water. All the studies demonstrate that the most ubiquitous compounds are the ones that are most consumed by the population (e.g. analgesics, anti-inflammatories, lipid regulators, antibiotics). Moreover, treated wastewater effluents have been found to be the main cause of water contamination with PPCPs, and in most of the cases the samples collected from sampling points downstream of WWTPs were contaminated with these compounds, although their concentrations varied and depended mainly on the extent of water dilution, and therefore on water flow.

2.4.3. Surfactants

Surfactants are one of the groups of organic chemicals with highest production rates, increasingly being used all over the world. They are used, not only as industrial and domestic detergents, but also as emulsifiers, paints, antifoamers or pesticide adjuvants.

After usage, surfactants are discharged into municipal sewer systems and then treated in WWTPs where they are completely or partially removed, being present in the effluents discharged into surface waters. Therefore, their main introduction into the environment is via wastewater discharges.

The Llobregat River receives wastewaters form several textile and tannery industrial plants mixed with municipal wastewaters. Due to this fact, several studies have been performed to determine the presence of surfactants and their degradation products in the receiving media.

During the wastewater treatment some of these surfactants biodegrade to non-toxic compounds before reaching the environment, but alkylphenol ethoxylates (APEO) deserve particular attention due to the endocrine disrupting potential of their degradation products, confirmed by numerous in vitro and in vivo studies. Therefore, most of these studies focus on the presence of these compounds (Brix et al., 2010; Cespedes et al., 2005; Diaz et al., 2002; Diaz et al., 2002; Gonzalez et al., 2004; Petrovic et al., 2002; Petrovic et al., 2002; Sole et al., 2000). They have been reported in the entire river basin (see Table 2.2) downstream of the WWTP, reporting a general trend of increasing analyte concentrations from the upper to the lower course of the river, with the highest concentrations in the Rubí Creek. The concentrations ranged from a few µg/L to several hundred µg/L with a general trend to decrease as reported for the WWTP located in this area by González et al. (2004). This decrease is probably due to the restriction in use of these compounds in household detergents and a progressive substitution by more degradable ones like AEO. This decrease in alkylphenolic concentrations compared with the previous studies suggests an improvement of the environmental status due to the decrease of the presence of Endocrine Disrupting Compounds (EDCs) in the river.

Although the alkylphenolic compound found in higher concentrations was nonylphenoxy dicarboxylate (NP2EC), nonylphenol (NP) concentrations detected in some studies exceed the maximum allowable concentration (MAC) of 2 µg/L established by the EU directive on Priority Substances (Directive 2008/105/EC).

Due to their importance a more detailed study was carried out in the Sant Joan Despí DWTP where samples from each treatment step were taken to evaluate the degradation of nonylphenolic compounds and the formation of brominated and chlorinated derivatives during the drinking water treatment process (Petrovic et al., 2003). These compounds were detected at low µg/L, with NP2EC also being the most abundant compound with concentrations below 15 µg/L.

Other surfactants like sulfophenylcarboxylates (SPCs), the degradation products of linear alkylbencene sulphonates (LAS), were also determined in the intake of Sant Joan Despí DWTP reporting average concentrations of 5 µg/L, where C8-SPC and C9-SPC were the most prominent homologues (Eichhorn et al., 2002).

Moreover, alcohol ethoxylates (AEO), coconut diethanol amides (CDEA) and LAS were detected at the mouth of the Llobregat River (Gonzalez et al., 2004; Petrovic et al., 2002) with concentrations ranging from 0.93-92 µg/L for LAS, up to 3.9 µg/L for AEO and 0.13-4.2 µg/L for CDEA.

Table 2.2. Contaminants and biological effects observed in the Llobregat River

Sampling point	Target compound	Concentration (min - max)	Bioassay	Biological effect observed	Reference
Mouth of the river	AP, NPEC, NPEO	<LOD-4.8 µg/L	-	-	(Petrovic et al., 2002)
Mouth of the river	AP, NPEC, NPEO	<LOD-0.52 µg/L	-	-	(Gonzalez et al., 2004)
Intake DWTP	NP, NPEC, short NPEO	0.7-16 µg/L	-	-	(Diaz et al., 2002)
Intake DWTP	NP, short NPEO	0.51-3.8 µg/L	-	-	(Diaz et al., 2002)
Intake DWTP	NP, NPEC, short NPEO	1.1- 15 µg/L	-	-	(Petrovic et al., 2003)
Intake DWTP	Oestrogens BPA	<LOD-0.022 µg/L 0.065-0.295 µg/L	-	-	(Rodriguez-Mozaz et al., 2004)
Intake DWTP	Oestrogens	0.33-0.68 ng/L	-	-	(Rodriguez-Mozaz et al., 2004)
Low part of Llobregat Basin, WWTP influents and effluents	AP, BPA, phthalates	<LOD- 37300 µg/L for surface waters <LOD- 44800 µg/L for WWTP influents <LOD- 10600 µg/L for WWTP effluents	Recombinant Yeast Assay (RYA)	Weak oestrogenic activity in surface water samples (except for two points). High values in influent water but removed (70-90%) compared to effluents.	(Cespedes et al., 2005)
Low part of Llobregat Basin	Metals	<LOD – 6 000 µg/g dw	Antioxidant enzyme activities, phase II enzymes, lipid peroxide levels	Antioxidant activities increased with metal content downstream	(Barata et al., 2005)
Low part of Llobregat Basin	Pesticides	Up to 785 ng/L	Diatoms, invertebrates, biofilms	Changes in diatoms distribution; Structural and functional aspects of biofilm	(Ricart et al., 2010)
Anoia River	(presence of AP reported from other studies)	Not specified	VTG in plasma and liver, estradiol, testosterone, xenobiotic metabolising capacity in liver	Highly variable content of VTG and an enhanced xenobiotic metabolism in the affected fish group	(Sole et al., 2003)
Anoia River	(presence of AP reported from other studies)	Not specified	VTG mRNA liver concentration in male carps	Presence of VTG mRNA	(Garcia-Reyero et al., 2004)
Anoia and Cardener River (downstream WWTP discharges)	NP, NPEO Oestrogens and progestogens	<LOD- 644µg/L	Vitollegin (VTG) plasma concentration in male carps	Increasing levels of VTG in male carp related to NP concentration	(Sole et al., 2000)

Table 2.2. Contaminants and biological effects observed in the Llobregat River (cont.)

Anoia and Cardener River (downstream WWTP discharges)	NP, NPEO Oestrogens and progestogens	<LOD-644 µg/L	Recombinant Yeast Assay (RYA)	Lower oestrogenic response than the observed in WWTP except in Manresa WWTP discharge.	(Garcia-Reyero et al., 2001)
Anoia and Cardener River	AP, Oestrogens and progestogens	<LOD- 35 µg/L for surface waters <LOD- 820 µg/kg for sediments	Vitollegin (VTG) plasma concentration in male carps	Correlation between VTG and AP in water and sediments samples and for E3 and E1 in water (r>0.78)	(Petrovic et al., 2002)
Anoia and Cardener River	Organochlorinated compounds, PAHs	0.1 – 85.4 ng/g dry weight	Biological markers: cytochrome P450, Phase II enzymes, EROD activity	Activities increased in sampling points downstream a WWTP	(Fernandes et al., 2002)
Anoia and Cardener River, Rubí Creek, WWTP effluents	Pharmaceuticals	<LOD- 8.8 µg/L for surface waters <LOD- 85 µg/L for WWTP effluents	*Vibrio fischeri* bioluminescence inhibition	Up to 36.8% in surface waters 20.6–55.8% in effluents	(Farre et al., 2001)
Along the river	General chemical paremetres	-	Diatoms	Variance in species composition	(Sabater et al., 1987)
Along the river	General chemical paremetres	-	Diatoms	Variance in species composition	(Leira and Sabater, 2005)
Along the river	General chemical paremetres	-	Diatoms	Variance in species composition	(Tornes et al., 2007)
Along the river	Metals, pesticides, PAH's, etc.	Phenanthrene < 85 ng/L; Tertbutylazina < 2140 ng/L; diazinon < 2826 ng/l; t-octylphenol <448 ng/l; Zn < 37 µg/L	*Daphnia magna*, macroinvertebrate community	Decreased in *D. magna* post-exposure feeding rates; decrease in riparian habitat indexes	(Damasio et al., 2008)
Along the river	Pharmaceuticals	Range 1-10 ng/L	Invertebrates and diatoms	Invertebrate abundance and biomass	(Muñoz et al., 2009)
Along the river	Pesticides, surfactants, pharmaceuticals, etc.	Not specified	Autotrophic biofilm communities	Diversity decreased as concentration of Diuron increased	(Ricciardi et al., 2009)

AP: Alkylphenols; NP: nonylphenol; NPEC: nonylphenol carboxylate; NPEO: nonylphenol ethoxylates; E3: estriol; E1: estone; BPA: Bisphenol A; PAH: Polycyclic Aromatic Hydrocarbons

2.4.4. Oestrogens and progestogens

The main source of natural and synthetic oestrogens and progestogens into the aquatic environment is anthropogenic after their usage for the treatment of certain hormonal disorders (e.g. menopause) and cancers and in birth control pills.

The elimination during wastewater treatment can vary depending on the compound and the type of treatment. Furthermore, less active conjugated forms in which oestrogens are excreted can be deconjugated by microorganisms during water treatment discharging the parent compound into the effluents (Petrovic et al., 2004).

Representative oestrogens, both natural and synthetic, progestogens and the natural hormone progesterone were evaluated at several points in the Cardener and Anoia tributaries but were detected below the detection limit (Cespedes et al., 2005; Sole et al., 2000).

In recent years and due to the use of novel techniques that permit the determination of oestrogens and progestogens in a low ng/L range, the detection of these compounds has been possible at several points in the Llobregat River and its tributaries within this range.

The steroids most frequently monitored along the Llobregat River are oestriol, oestrone and oestrone sulphate, the main metabolites of estradiol (Brix et al., 2010; Kuster et al., 2008; Lopez-Roldan et al., 2010; Petrovic et al., 2002; Rodriguez-Mozaz et al., 2004; Rodriguez-Mozaz et al., 2004), not surpassing 25 ng/L in any case. The highest concentrations were found in one channel receiving water from Anoia River, the Rubí creek and Sant Feliu WWPT to by-pass it downstream of the DWTP and near Castellbisbal, an area influenced by urban activities.

The steroids found in the Llobregat are the ones with lower oestrogenic potential. Contrary, the natural hormone estradiol and the synthetic ethinyl estradiol, the compounds with higher oestrogenic potential were not detected in the aforementioned studies. The concentrations found can pose a risk to aquatic organisms given that a concentration of 1-10 ng/L is considered enough to cause oestrogenic effects (Petrovic et al., 2002).

2.4.5. Other compounds

Other groups of compounds have been also of concern mainly due to their characteristics. Bisphenol A and phthalates, used as plasticizers, were studied due to their endocrine disrupting potential (Cespedes et al., 2005; Rodriguez-Mozaz et al., 2004), reporting concentrations of up to 2.97 µg/L and 6.85 µg/L, respectively, with diethyl phthalate (DEP) being the compound found in higher concentrations.

Polycyclic aromatic hydrocarbons (PAH) are another group of concern due to their mutagenic, carcinogenic and teratogenic characteristics included in the list of priority substances of the WFD that have been studied in the Llobregat basin. PAHs sources are mainly industrial due to the combustion of fossil fuels, and natural combustion such as forest fires. Pyrene, fluoranthene and phenanthrene were studied over a long time period (2003-2006) found in maximum concentrations of 16 ng/L (Terrado et al., 2009). In a study carried out after extensive forest fires occurred in 1994 (Olivella et al., 2006) twelve parent PAHs were detected along nine sampling sites in the Llobregat. One month after the forest fires the total PAH varied from 2 to 336 ng/L whereas after heavy rainfalls the concentration decreased by a factor of three.

Regarding inorganic compounds, although most of studies have been carried out on sediments, there are some published results on surface waters along the Llobregat basin (Castillo et al., 2001; Fernandez-Turiel et al., 2003). High contents of Na, K, Mg, Cl, Br, Rb and Sr have been detected related to mining and industrial activities from potash exploitation. Also, industrial and residential uses located in the lower part of the river basin result in increases of P, B, Mn, Fe, Pb, Al, Cr, Co, Ni, Cu, Zn, As and Sb concentrations in the river water.

Several compounds causing odour and taste events in drinking water have also been investigated in Llobregat River waters due to the public perception problems that these compounds can cause in consumers and therefore in water suppliers. Creosote, a raw material used in wood-preserving factories and mainly composed of PAH, was identified as the compound causing odour episodes in the river. After a change in practices by the wood-preserving companies, no creosote episode has been detected in the river (Ventura et al., 1998). Geosmin and odour natural compounds causing an earthy-musty odour in water were detected in concentrations between 50-150 ng/L achieving a good elimination along the drinking water treatment, but still being present in drinking water (Romero and Ventura, 2000). 2-3 butanedione (diacetyl), an organic compound imparting sweet and buttery odour problems was detected at the intake point at a concentration of 0.9-26 µg/L, identifying this compound as the cause of several odour events (Diaz et al., 2004). Trichlorobromophenols, suspected to be responsible for unresolved taste and odour in water, were also studied in raw and treated water in Llobregat reporting concentrations in river below 400 ng/L but the sum for all studied compounds reached 2.1 µg/L. In contrast, the

concentrations found in treated water were near the quantification limits but were still candidates for unresolved cases of taste and odour (Diaz et al., 2006).

The presence of other group of compounds, the chlorinated toluenes, coming from the textile industries and inefficient treatment of wastewaters, was evaluated over a year (2003-2004). Weekly sampling reported concentrations at a low ng/L after treatment in WWTP (Marti et al., 2005).

2.5. Biological effects

It is known that chemicals may pose hazards to organisms including humans, as indicated by observable effects (e.g., in vivo and in vitro bioassays). Being aware of the presence of these contaminants in the river, identifying links between water quality and the biological effects observed has been the objective of many studies (see Table 2.2) and will continue to be so in future in order to discover the ecological status of water bodies as required in the WFD. These kind of ecological status studies are difficult to assess, since evaluation methods are not standardised.

The application of bioassays indicating effects on cellular, organism or population level in laboratory test systems and linking measurable effects of complex environmental samples to distinct toxicants are required to bridge the gap between chemical contamination and ecological status (Brack et al., 2007).

Muñoz et al. (2009) have used multivariative techniques to determine potential relationships between the presence of pharmaceuticals and the structural composition of biological communities (diatoms and invertebrates) finding a potential causal association between the concentration of some anti-inflammatories and β-blockers and the abundance and biomass of several benthic invertebrates. Additionally, hazard quotient (HQ) indexes have been estimated as the ratio between concentrations and EC50 (50% effect concentration) reported values for three bioassays commonly used: fish, Daphnia and algae, calculated as the sum of the HQs for all the compounds. In general, HQ tend to increase when going downstream and only points located farther upstream can be qualified under low risk for the three bioassays (Ginebreda et al., 2010).

Multivariative techniques have also been used to study the effects of pesticides in benthic algae and invertebrate fauna communities revealing a potential relationship between triazine-type herbicides and the distribution of the diatom community (Ricart et al., 2010).

Leira and Sabater (2005) were aware that distribution of diatom was influenced, not only by chemical factors but also by physiographical ones. Salinity, high nutrient concentration and low flow are

considered to be responsible for biologically poor communities made up of tolerant taxa. This trend in the modification of the distribution of diatom species along the Llobregat River according to different characteristics of each stretch of the river was also reported in other studies (Sabater et al., 1987; Tornes et al., 2007).

Although several studies have been performed so far, some challenges still exist that need to be overcome to achieve an optimal characterisation of aquatic systems, by combining chemical analyses and biological-effect assessment into a single strategy. Tolerance of organisms towards toxicants is not linear in natural environments and the interactions that occur within a population are not considered in toxicity assessment. Moreover, making estimations and predictions from relatively limited laboratory data to real ecosystems is problematic and gives rise to some degree of uncertainty (Geiszinger et al., 2009). In this sense, a combination of different taxa was used for studying relationships between chemical pollution and loss of diversity. In the Llobregat River, autotrophic biofilm diversity was observed to decrease as concentration of diuron increased when combining several diversity indices while no significant variation was observed in the conventional ones, concluding that diuron may influence only certain levels of taxonomic classification (Ricciardi et al., 2009).

The effects of glyphosate on the structure and function of the Llobregat River ecosystem after their field application has been studied (Puertolas et al., 2010). Due to the fact that the macroinvertebrate communities were dominated by taxa tolerant to pollution, the herbicide application did not affect the abundance or number of taxa in any location studied. Nevertheless, significant specific toxic effects on transplanted *Daphnia magna* and field collected Hydropsyche exocellata were observed. Effects included *Daphnia magna* feeding inhibition and oxidative stress related responses such as increased antioxidant enzyme activities related with the metabolism of glutathione and increased levels of lipid peroxidation.
Daphnia magna also has been used in sensitive, robust and ecological methods aimed to diagnose sublethal effects of toxic effluents rich in metals and agrochemicals (Damasio et al., 2008). Feeding rates were negatively affected by the presence of PAHs and positively affected by nitrogen sources (NO3, NO2). Additionally, temperature, suspended solids and triazines contributed marginally to observed responses.

Pharmaceutical compounds were analysed in the same area, also including samples from Rubí Creek and effluents from WWTPs (Farre et al., 2001). Toxicity values were obtained by measuring bioluminescence inhibition of *Vibrio fischeri* bacteria. Although positive values were found in most of the samples (up to an inhibition of 36.8%), the contribution of pharmaceuticals in global toxicity was supposed to be low so they can only be considered as tracers.

As a key conclusion reported in several articles, further studies are needed on the usefulness of in-situ bioassay responses in detecting subtle effects on aquatic organisms exposed chronically to multiple environmental factors and/or to low levels of contaminants (Maltby et al., 2002).

Concerning the oestrogenic effects that pollutants may pose to organisms, EDCs are defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock et al., 1996). EDCs are a wide range of industrial and household chemicals which include persistent organic pollutants (e.g. alkylphenols and their ethoxylates, BPA, phthalates, pesticides), some heavy metals as well as steroid oestrogens which have caused major concern in the Llobregat River because of the potential risk to aquatic organisms.

The production of vitellogenin in males (also VTG, a yolk egg precursor synthesised naturally only in female fish) is a widely accepted measure of oestrogenicity. The VGT increase in plasma samples of male carps (Cyprinus carpio) was related to the presence of NP at sites close to WWTPs (Sole et al., 2000) and to alkylphenolic compounds in water and sediment samples and for oestriol and oestrone in water (Petrovic et al., 2002). Moreover, the levels of VTG mRNA in the liver of the same fish were attributed to the presence of NP derivatives in this river (Garcia-Reyero et al., 2004).

Carps collected in Anoia River were also fully characterised according to different paremetres (VTG in plasma, VTG in liver, estradiol, testosterone, xenobiotic metabolising capacity in liver) and classified into three groups: apparently normal males, apparently normal females and affected fish. Results showed a highly variable content of VTG in all groups and an enhanced xenobiotics metabolism in the affected fish group. An increase in VTG, sex hormones and most enzymatic activities from January to March was observed but attributed to higher water temperature. The study points out the need for further research on the reproductive capacity of affected fish (Sole et al., 2003).

Recombinant yeast assay (RYA) has been also used to determine the presence of EDC. Oestrogenic activity measured by RYA showed a good correlation with the chemical analyses performed. Clear positives were found only in the Anoia River (Garcia-Reyero et al., 2001) and in the lower course of the Llobregat (Cespedes et al., 2005) but showed much lower oestrogenic response than that observed in WWTPs. This activity was mainly attributed to the presence of NP with a minor contribution of BPA and nonylphenol monoethoxylates.

Other studies established a link between residues of selected pollutants in tissues of some organisms and biochemical responses. Although a general pattern can be established, that is, an increase in the activity of some biomarkers, such as EROD activity can be linked to a higher exposure to some organic pollutants

that can be found downstream at a WWTP, some inconsistencies are found showing that one biomarker is not enough to assess responses of organisms to contamination (Fernandes et al., 2002). The relationship has been also studied among residues of selected metals in Caddisfly larvae of Hydropsyche exocellata and some enzyme activity. Oxidative stress increased towards downstream locations where metal concentration is high and antioxidant enzymes respond to them. But it is also highlighted that other environmental factors may also alter this status (Barata et al., 2005).

2.6. Conclusions

In order to comply with the WFD and to ensure the good ecological status of all water bodies, a large number of compounds will have to be analysed. The methods used to monitor pollutants of concern very commonly involve spot sampling at specified periods of time from the field back to the laboratory where they are analysed by classical chromatographic and spectroscopic techniques. Almost all of the studies reviewed in this work belong to this type of monitoring but they are based on infrequent samples at a limited number of sampling points that may not provide a representative picture of water quality, because pollutants levels vary both spatially and temporally Therefore, the cost of obtaining representative data of the overall water quality using these methods would be high.

Furthermore, to obtain an accurate picture of the situation and to be able to understand the ecosystems and how to preserve and enhance them, it is not enough to measure whether the pollutants concentration is below the limits established by legislation or not, because this is not necessarily representative of the water status. Pollutant monitoring should be complemented by studies dealing with the determination of the effects caused by the contamination. Studies showed that although some correlations between the concentration of target contaminants and specific alterations in some organism can be established, a comprehensive battery of biological tests needs to be performed to obtain a complete characterisation of the ecosystem status, as different taxa of living species have different reactions to specific pollutants. In this aspect further studies are needed in order to discover the ecological status of the Llobregat River as required by the WFD.

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3

On-line bacteriological detection in water

3. On-line bacteriological detection in water

3.1. Abstract

Microorganism contamination is a permanent concern in a wide range of fields, including the water treatment, food and pharmaceutical industries, in which fast detection is critical to prevent microbial outbreaks.

Concerning water monitoring, current procedures for water quality analysis are based on periodic sampling and detection by culture methods. These methods are slow, requiring 24-48h for completion, meaning that when the first results reach the decision-makers and an alarm is triggered, exposure time has been already significant and the population may have been exposed to a health hazard.

There is a need for rapid and reliable detection of contaminants across a broad spectrum of water management situations. For real-time detection, online monitoring seems to be the ideal approach, but the need to adjust the available techniques for autonomous operation and the optimisation of the time of response is a substantial challenge.

This review presents the findings of an identification study about the state-of-the-art of technologies and commercial devices for on-line bio-monitoring of water quality, specifically for the detection of faecal contamination. We have also included studies dealing with the verification or use of these devices.

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3.2. Introduction

In the water treatment and supply industry, transmission of diseases can be related to inappropriate treatment methods, a failure in operation and supervision, or shortcomings in quality monitoring [1]. In fact, it can theoretically be argued that all waterborne diseases can be prevented by appropriate monitoring and corrective measures taken in good time [2]. Waterborne diseases are typically caused by enteric pathogens, mainly transmitted by the faecal-oral route, such as bacteria (*Salmonella spp, Shigella spp, pathogenic Escherichia , Campylobacter spp, Vibrio cholerae* and *Yersinia enterocolitica*), viruses (Hepatitis A and E, enteroviruses, adenoviruses, small round structured viruses including Norwalk virus, astro and rota viruses) and protozoa *(Entamoeba histolytica, Giardia intestinalis, Cryptosporidium parvum*). Other opportunistic pathogens found in water promote infections of the skin and mucous membranes of the eye, ear, nose and throat (*Pseudomonas aeruginosa, Aeromonas*, and species of *Mycobacterium*) or infections contracted by the inhalation of contaminated aerosols: *Legionella spp* (legionellosis), *Naegleria fowleri* (primary amoebic meningoencephalitis) and *Acanthamoeba spp* (amoebic meningitis, pulmonary infections).

Since analysis and detection of all these pathogens is highly unfeasible, there is an established selection of the most suitable to be used as indicators. Currently, *E.coli* is used as an indicator of water safety regarding faecal contamination in almost all water quality legislation in the world for drinking water and bathing waters. *E. coli* is a bacterium that lives in high numbers in the intestines of warm blooded animals. Its value as an indicator of faecal contamination in water has been proved on numerous occasions [3].

Nowadays, the analysis of bacteriological quality is mainly performed in the laboratory by culture methods. However, culture methods have a variety of drawbacks. A primary concern is the time between sample collection and result reporting (>18 h), creating a risk that humans will be exposed to contaminated water. In addition, standard culture methods do not provide species-level identification that would provide a better indication of faecal contamination [4]. Recently, researchers have increasingly moved toward molecular technologies to meet the need for rapid, multiplexed, species-level detection [5]. Techniques such as polymerase chain reaction-based methods (PCR), have been proved to provide sensitive, rapid, and quantitative analytical tools for pathogen determination [6].

In recent years, concerns have risen about the need for on-line monitoring of water systems given that existing laboratory-based methods are too slow to develop operational responses and do not provide a level of public health protection in real time. This need for real-time monitors should be assessed on a

case-by-case basis based on the requirements of an individual water management body. Water utilities worldwide therefore employ on-line monitoring tools and early warning systems at all stages of the urban water cycle to measure physical properties and some chemical compounds, through intake protection, treatment operations and distribution systems [7].

In the distribution network, the challenge is to establish a rapid alert and response to a contamination event in order to minimise the population at risk. If an incident is detected early enough, emergency procedures can easily and vastly limit damaging effects [8, 9]. Therefore, accurate real-time detection of CBRN contaminants is often required for planning and implementing mitigation measures to protect water supplies [10].

The development of autonomous methods for the online measurement of microbiologic paremetres requires the automation of the analytic methodology for fast analysis of faecal indicators and the design of instruments to operate in real environments. In this regard, we have performed a review of existing technologies and instruments (commercial and prototypes) that have tried to deal with this challenge.

3.3. Review of analytical techniques

The range of methods available for the application of molecular techniques has increased, and the costs involved have fallen. Additionally, recent improvements in detection technologies have allowed the simultaneous detection of multiple targets in a single assay. However, the analytical techniques available today and those under development require further refinement in order to be standardised and applicable to a diversity of matrices [6]. Very recently, attention has also been focused on nanobiotechnologies for food-borne pathogen detection in food matrices and environmental matrices [11].

Many emerging biological sensors rely on the detection of specific biomolecules, including adenosine triphosphate (ATP), enzymes and other proteins, as well as immunoassay and polymerase chain reaction (PCR) techniques. The major limitation of these and other biological systems lies in their inability to detect low concentrations of microorganisms, which unlike chemicals are not uniformly distributed in aqueous environments. Other biological sensors rely on the optical properties of water and the analytes present in the sample, and include those based on evaporative light scattering detection, refractive index measurement, fluorescence detection, and Raman spectroscopy [7].

The techniques reviewed have been used to evaluate the microbiological quality of water or they have been applied to other fields but could be potentially used for water applications. As the ultimate objective of this review is real-time detection, we have selected techniques according to their potential for on-line applications.

3.3.1. Light Scattering

Light scattering technology is a simple scanning procedure that provides information about the presence of particles of a certain size. When a laser beam is sent through flowing water, the laser light is scattered at right angles by the presence of particles in the water. Optical devices such as photodiodes collect the scattered light, which can be analysed to determine the size and number of particles present in the water sample.

However, the technology does not allow for the determination of specific information about the identity of the particle; furthermore, it can only detect particles within a certain size range. The two main challenges facing this technology are the decrease in the number of false positives and the improvement of the level of sensitivity. Measurements of particle volume for specific particle sizes are site-correlated with the absolute numbers of a particular pathogen [12].

The new Multi-Angle Light Scattering (MALS) technology uses optical fingerprints in order to identify the different types of bacteria. The optical signatures obtained are compared with a database, and particles are classified into shape categories [13, 14]. Figure 3.1 shows a diagram of the functioning of this technology.

An AWWARF research project [15] determined that MALS technology can distinguish between Cryptosporidium and finished water matrix particles at a level high enough to serve as an early warning tool for water systems. The identification rate of *Cryptosporidium parvum* oocysts varied from 11% to 45%, and false-positive rates varied from 0.3% to 3%.

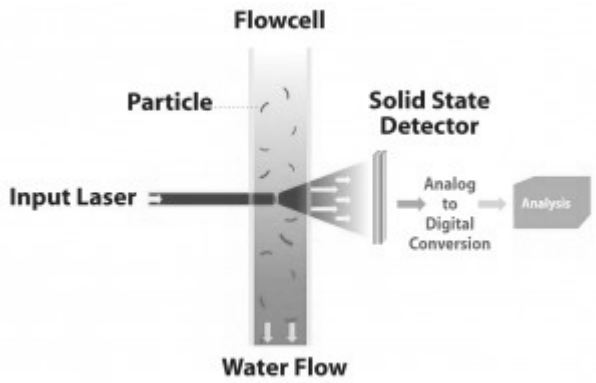

Figure 3.1. Multi-Angle Light Scattering (MALS) Technology (source: www.jmar.com)

3.3.2. ATP Luminescence

A common indicator for microbial presence widely used in the food and beverage industry is ATP luminescence. For the measurement of microbial ATP, cells in the water sample are lysed to release ATP into the solution. The reaction is catalysed by luciferase, which breaks down ATP and releases a photon of light from luciferin. Then an illuminometer reads the quantity of light emitted from the reaction. The light intensity is directly related to the concentration of ATP in the sample. It is important that the routine testing establishes a baseline trend for ATP results, and then subsequent fluctuations in ATP can indicate a change in microbial status of the system [16]. Provided that the concentration of ATP depends on the specie, the specific strain, and the environmental and metabolic factors, ATP is only an approximate indicator of the biomass in the sample. In the food and water industry, ATP technology is used for the rapid enumeration of total viable counts [17]. Figure 3.2 shows a representation of an ATP Luminescence measurement.

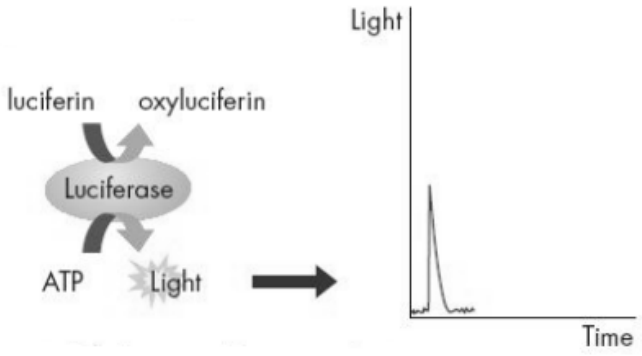

Figure 3.2. ATP Luminescence measurement (source: www.qiagen.com)

3.3.3. Immunoassays

The principle behind rapid immunoassay technologies is the detection of the antigen-antibody reaction. The presence of a microbial contaminant is detected when specific antigen proteins in the sample bind with the corresponding antibodies. Classic examples of immunoassays are the enzyme-linked immunosorbent assay where a secondary antibody is conjugated to an enzyme that forms a coloured precipitate (ELISA), and the enzyme-linked fluorescent immunoassays that give off light (ELFA). Immunosorbent refers to the immobilisation of the capture molecules on a surface, such as a membrane.

The target molecules in the sample are antibodies if antigens are immobilised and antigens if antibodies are immobilised [18].

A test showed that immunomagnetic separation plus PCR and RNA techniques presented great promise in their ability to completely automate the detection of pathogens [19]. Setterington and Alocilja [20] detected *E. coli* by isolating cells by immunomagnetic separation, labelling them with electroactive polyaniline, and detecting them by cyclic voltammetry on screen-printed carbon electrodes. Initial results showed a detection limit of 70 CFU/ml with a linear range of 101 to 105 CFU/ml. The assay required 70 min from sampling to result. The experiment developed by Laczka [21] coupled magneto-immunocapture and amperometry at flow channel microband electrodes. Experiments with *E. coli* evidenced a linear response for concentrations ranging 102-108 CFU/ml, without preenrichment steps. The whole assay could be completed in 1h. Immunomagnetic beads efficacy has been tested for the analysis of *E. coli* by [22]. In inoculated food samples, the technique detected 1 CFU/g of *E. coli* after a 6-hour enrichment at 37 °C.

3.3.4. Polymerase Chain Reaction (PCR)

The PCR technique is a three-step cyclic in vitro procedure based on the ability of the enzyme DNA polymerase to copy a strand of DNA. The region of DNA to be amplified is specified by the choice of primers. Primers are short oligonucleotides, usually 20-30 nucleotides in length, whose sequence matches the end of the region of interest. Amplification takes place over a number of thermal cycles. In subsequent cycles, primers will bind to both the original DNA and the newly synthesised DNA resulting in an exponential increase in the numbers of copies. The results of PCR are detected by the use of fluorescent double-stranded DNA dyes or probes [16].

PCR is a highly sensitive, specific and potentially rapid detection method. However, the potential of this technique for on-line environmental monitoring is limited because of the use of disposable prepared kits for commercial PCR instruments. This greatly increases mechanical complexity, because sophisticated robotic mechanisms must replace the disposable elements [23]. The relationship between PCR quantification and culture-base methods has been successfully used to quantify faecal indicator bacteria at coastal and some inland water sites [24, 25]. Another comparison for detection of *Legionella pneumophila* showed that real time PCR methods offer the benefit of speed over traditional culture methods [26].

To detect several organisms in a single reaction, the simultaneous amplification of more than one locus is required. This methodology is referred to as multiplex PCR in which several specific primer sets are

combined into a single PCR assay. Monitoring bacterial faecal contamination in waters using multiplex real-time PCR assay for *Bacteroides spp*. and faecal *enterococci* was carried out by Agudelo [27]. Detection levels were much higher and the procedure could be performed in less than 3 h.

In a similar way to PCR approaches, a highly specific real-time NASBA (nucleic acid sequence-based amplification) method could also be used for rapid detection of viable *E. coli* in water samples [3, 28]. The sensitivity of the NASBA assay was comparable with the culture method and approaches a sensitivity of 1 CFU/100ml. Results were obtained in only 3–4 h, enabling same-day responses to faecal contaminations.

3.3.5. Enzymatic fluorescence techniques

The recognition of colonies of presumptive target organisms has been facilitated by the introduction of chromogenic and fluorogenic media. The microbiological growth media contains enzyme substrates linked to a chromogen (colour reaction), fluorogen (fluorescent reaction) or a combination of both. The target population is characterised by enzyme systems that metabolise the substrate to release the chromogen/fluorogen. This results in a colour change in the medium and/or fluorescence under long wave UV light.

Most encountered devices using this technology are based on IDEXX Colilert®, which is used for the simultaneously detection and enumeration of total coliforms and *E. coli* in water and wastewater based on the Most Probable Number (MPN) principle. This method uses two chromogenic nutrient indicators: ortho-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG) as the major sources of carbon. As coliforms grow, they use β-galactosidase to metabolise ONPG and change it from colourless to yellow. *E. coli* uses β-glucuronidase to metabolise MUG and create fluorescence [29].

A published study reported the detection of *E. coli*based on the hydrolysis of chemiluminescent 1,2-dioxetanes. Chemiluminescence showed greater sensitivity than fluorescence. Combined with membrane filtration, this shows potential for early-warning detection of microbial contaminations in drinking water (4–6 h) [30].

The M.E.R.® ("Méthode Enzymatique Rapide") approach is a rapid quantification test of *E. coli* presented in a marine or fresh water sample [31]. This enzymatic test allows the detection of a specific enzyme activity as the β-D-glucuronidase for *E. coli* and was also adapted to the detection of the *Enterococcus spp.* specific β-D-glucosidase activity. Research on this technology showed that

assessments of water given by the M.E.R. and the ISO standard methods were in relatively high agreement for the quantification of *E.coli* and *Enterococcus* in marine waters [32, 33].

3.3.6. Fluorescent in-situ hybridisation (FISH) techniques

Fluorescence in-situ hybridisation (FISH) with ribosomal RNA (rRNA) targeted oligonucleotide probes is the most commonly applied technique among the 'non-PCR based' molecular techniques. The choice to target RNA instead of DNA results in a more sensitive technique (higher copy numbers available) and a link to viability. In the preparation of FISH, microbial cells are treated with appropriate chemical fixatives and then hybridised under stringent conditions on a glass slide or in solution with oligonucleotide probes [16]. After stringent washing, to remove unbound probe, specifically stained cells are detected via epifluorescence microscopy. FISH has been applied with this aim for the detection of emerging pathogens from water, sewage and sludge [34].

An integrated microfluidic device (µFlowFISH) capable of performing fluorescence in-situ hybridisation (FISH) followed by flow cytometric detection for identifying bacteria in natural microbial communities was tested. The device was used for the detection of species involved in the bioremediation of Chromium (VI) and other metals in groundwater samples. The µFlowFISH approach provided an automated platform for the quantitative detection of microbial cells from complex samples with low cell numbers [35]. The feasibility of using real-time potentiometric detection of bacteria in complex samples was proved by Zelada-Guillén [36]. The potentiometric biosensor used carbon nanotubes chemically linked to aptamers as probes to selectively detect and identify a particular strain of *E. coli* in real complex samples in a few minutes.

3.3.7. Molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers (MIP) are polymers that have been processed using the molecular imprinting technique which leaves cavities in polymer matrix with an affinity to a chosen molecule. Those synthetic receptors can be designed for a range of toxins and some microorganisms. MIPs have greater stability, being able to withstand climate extremes and larger sensitivity ranges, than antibodies. Some analytes for which MIPs have been developed include the algal toxins, domoic acid, and microcystin, and the fungal toxins, aflatoxin B1, and ochatoxin A [18]. Figure 3.3 shows the preparation of molecularly imprinted data.

A novel, affinity-augmented, bacterial spore-imprinted, bead material was synthesised and evaluated using spore-binding assays with either *Bacillus thuringiensis* or *Bacillus subtilis* spores [37]. A MIP-based biosensor has been used for detection of the plant pathogen *Tobacco mosaic* virus at 100 ng/ml, thereby illustrating the potential of MIPs as specific receptors [38].

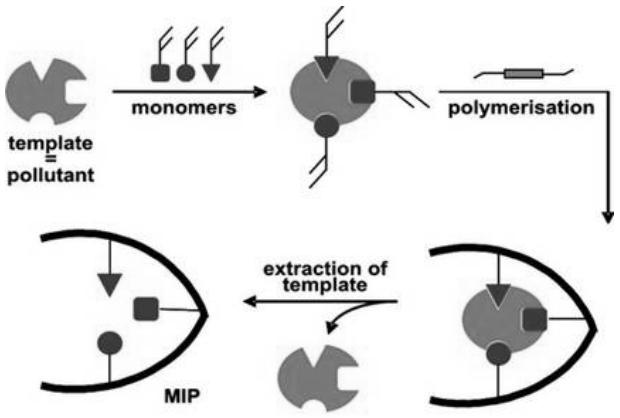

Fig.3.3. Preparation of Molecularly Imprinted Data (source: www.vti.bund.de)

However, some of the disadvantages of MIPs are the difficulty to completely remove the template from MIPs, the insolubility of the imprinted polymer and, although the polymer contains many imprinted cavities, only some of them match the template molecule [39].

3.3.8. Electrochemiluminescence (ECL) detection

ECL assays employ labels that emit light when electrochemically stimulated at an electrode. Attaching these labels to biological binding reagents allow their use in solid phase binding assays, such as nucleic acid hybridisation assays of sandwich immunoassays. The existence of small, stable and highly efficient ECL labels makes the technique robust, sensitive and easy to implement. Assays are carried out in disposable multi-well plates having integrated carbon ink electrodes. These electrodes act as solid phase supports for arrays of biological reagents and also provide the source of electrical energy for generating ECL signals. The use of array-based multiplexing allows for the detection of multiple analytes in each well of a multi-well plate (up to 25 per well of a 96-well plate). Usually a cooled CCD camera and molded lens image the ECL generated from the plate arrays.

ECL combined with the polymerase chain reaction (ECL-PCR) method was applied for the first time to the rapid detection of *Vibrio parahaemolyticus* in infected and uninfected sea foods [40].

Immunomagnetic separation (IMS) methodology was coupled to ECL detection to produce rapid detection (<1 h) of as few as 100 bacterial spores [41] or 1000 cells/ml of *E. coli* O57 and *Salmonella typhimuri* [42]

3.3.9. Raman Spectroscopy detection

Raman spectroscopy measures the inelastically scattered light following excitation. Figure 3.4 shows a representation of Raman spectroscopy. The advantages of Raman spectroscopy are significant for aqueous samples, as the infra-red absorption of water is avoided and spectral bands are generated more sharply and distinguishable [43]. Biological molecules such as nucleic acids, protein, lipids, and carbohydrates all generate specific Raman spectra, which provide biochemical information regarding the molecular composition, structure, and interactions in cells [44]. Therefore, from the whole-cell spectra, single microorganisms can be identified and discriminated. Raman spectroscopy has been further developed into two technologies: Surface Enhanced Raman Spectroscopy (SERS) for the identification from the spectra produced at the surface of the organism which has reacted with antibodies; and Laser Tweezer Raman Spectroscopy (LTRS), producing an optical "tweezer" to catch a microorganism and then laser light is used to produce a unique Raman spectrum that can be used to discriminate between different strains of bacteria [45]. Using this technique the discrimination between different strains of bacteria (*Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Streptococcus pyrogenes, Enterococcus faecalis* and *Streptococcus salivarius*) [42] and the germination of single *Bacillus* spores [46] have been reported.

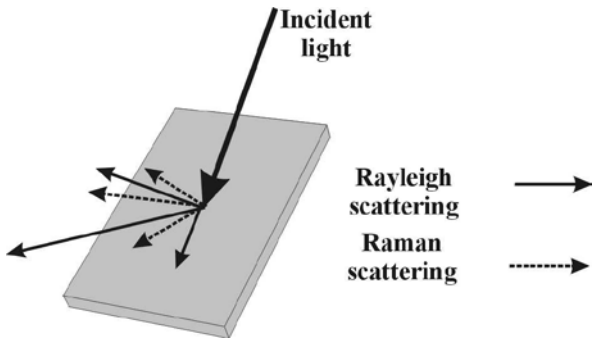

Fig.3.4. Representation of Raman Spectroscopy (source: www.porous-35.com)

3.3.10. Dye-loaded microspheres detection

Each microsphere can be coated with a separate capture molecule which can be involved in nucleic acid hybridisation, antibody recognition, a receptor-ligand reaction, or an enzymatic reaction. The reaction

times are about three times faster than standard microarrays because the microspheres in solution have 3-D exposure allowing for almost solution-phase kinetics, whereas flat microarrays are limited by solid-phase kinetics [47]. The microspheres pass through the detection chamber single file and are optically measured, usually by flow cytometry, which quantitatively measures the optical characteristics of the spheres as they are presented separately in front of a focused light beam.

In a general overview, optical techniques present the advantages of being very fast and reagentless, but they have very low specificity and a high risk of detecting false positives. Immunoassays have low sensitivity and require preconcentration, but they are a good basis for optical sensing upon capture. Fluorescence techniques are the most reliable and maybe ready alternative to culture methods, but are by far the slowest methods. PCR is fast, sensitive and very specific, but is affected easily by inhibitors or other matrix interference and is difficult to integrate into an autonomous device. Other emerging technologies introduce some new solutions and modified approaches, but they still need further research (and especially validation) to be considered for standardised online use. An example of application of Dye-loaded microspheres for river and seawater using Luminex technology is presented by Baums et al [5].

Table 3.1 presents an enumeration of the techniques presented above with some summarised issues and capabilities. Additionally, Table 3.2 summarises the application reported.

3.4. Assessment of commercial devices

In Table 3.3, several devices for microbiological detection are presented. Some of them have been specifically designed for water analysis. Some others have been designed for other different applications, but the instruments are versatile enough for the detection of several bio-targets, including enteric pathogen bacteria in water samples. In general, all of them are able to detect indicators of faecal contamination. The selection of devices is not intended to be an exhaustive enumeration of biological instruments, since such approaches already exist [48] but a representative list of the available instruments for previously reported technologies that presumably may better suit online water analysis. As far as possible, devices are listed in ascending order, from the least automated (need for sample handling) to on-line autonomous devices.

Few studies have been reported dealing with the validation of these instruments. Some of them are very new and, in a large number of cases, results of the tests are confidential or performed by private companies or other institutions with no interest in publication of results. Nevertheless, the studies identified have been reported.

RAPTOR, a rapid and automated fiber-optic biosensor assay for the detection of Salmonella in sprout rinse water was developed by Kramer [49]. *Salmonella typhimurium* could be positively identified when seeds were contaminated with 50 CFU/g. This biosensor assay system has the potential to be directly connected to water lines within the sprout-processing facility and to operate automatically.

Table 3.1. Identification of technologies for detection of microorganisms according to their potential to be automatised

Name	Basis	Issues	Capabilities	References
Light Scattering	Laser beam plus particle size or shape	Low level of sensitivity (>10^3 CFU/ml) High risk of false positives	Does not require preconcentration Potential for on-line applications	[12]
ATP luminescence	Cell lysis, enzyme activity, illuminometer	ATP is an approximate indicator of biomass activity. Cannot differentiate between bacterial species. Non-microbial ATP may cause false-positives		[16, 17]
Immunoassays	Antigen-Antibody	Cross reactivity Developed for grab samples Fast but not quantitative Sample concentration needed Lack of necessary sensitivity	Combined with detection techniques (like fiber optic-based biosensor) gains potential for automated applications	[18–22]
Polymerase Chain Reaction (PCR)	DNA/RNA amplification, fluorescence detection	Natural interferences (soil-derived humic & fulvic acid). Sample concentration required	High specificity Lab devices or Portable systems (autonomous platforms still in development)	[16, 23, 25–27]
Enzyme fluorescence	β-glucuronidase and fluorescence generation β-galactosidase and yellow dye	Very low concentrations leading to weak enzyme response. Cultivation needed	Easy automation if used with an optic sensor. Detection of *E.coli* + total coliforms	[29–31]
Fluorescent in-situ hybridisation (FISH)	RNA	Enrichment steps are often required e.g. magnetic beads	Allows for the detection of viable but non-culturable forms.	[16, 34–36]
Molecularly imprinted polymers	Shaped polymers as synthetic receptors	Emerging technique	Greater stability than antibodies	[18, 57, 58]
Electrochemiluminescence (ECL)	Bio-labels that emit light when electrochemically stimulated at an electrode	Target capture by sandwich immunoassay. Requires good binding sites.	The electrodes act as solid phase supports for arrays of biological reagents. Multiplex adaptable.	[56, 57]
Raman Spectroscopy	Target binding surface Spectrum comparison	Might show problems with turbidity and highly contaminated mixtures. Analysis on only 0,5 µL with no concentration step	Very sensitive	[43–45]
Dye-loaded microspheres	Particles coated with capture molecules. Colour optically measured	Emerging for water analysis	Fast kinetics Can select specific antigens	-

Table 3.2. Results for the application of the reported techniques for bacteriological detection at water samples

Type of technology	Paremetres analysed in water samples	LoD	Analysis Time	Ref
Light Scattering	*Cryptosporidium* *E. coli*	1 oocysts/mL 1000 CFU/mL	60 min continuous	[15, 55]
ATP luminescence	Total microbial biomass	200 CFU/ml	5 min	[60]
Immunoassays	*Bacillus anthracis* *Legionella pneumophila*	100 000 CFU/mL 800 CFU/mL	15 min 180 min	[18, 61]
Polymerase Chain Reaction (PCR)	*E. coli, Enterococci* *Legionella pneumophila*	15 CFU/100 mL 100 genomes units/L	3 h 3 h	[24, 26]
Enzyme fluorescence	*E. coli*, total coliforms	50 CFU/100 mL	1 h	[32, 33]
FISH	*E.coli*	100-100 CFU /mL 10 CFU /100 mL	2 h 10 h	[62]
Molecularly imprinted polymers	*Bacillus*	not specified	1 day (plus beads synthesis)	[37]
Electrochemiluminescence (ECL)	*E. coli, Salmonella*	1000 cells/mL	1 h	[63]
Raman Spectroscopy	*E. coli*	1000 cells/mL		[45]
Dye-loaded microspheres	*E. coli*	1000 cells/mL	1 h	[64]

Successful detection above regulatory standards was also obtained using ultrafiltration plus detection of enterococci in recreational waters. RAPTOR was shown to be more thorough and faster than other methods (e.g. qPCR) but the detection limits were not low enough for bathing waters [50].

P-CAN Sensor from IBI is based on the CANARY concept, which stands for “Cellular Analysis and Notification of Antigen Risks and Yields” and uses an array of B cells, each specific to a particular bacterium or virus. The cells are engineered to emit photons of light when they detect their target pathogen [51, 52].

A BioSentry instrument using multi-angle light scattering (MALS) technology was tested by Adams [53]. A laser beam strikes particles as they pass through the beam, generating unique light scattering patterns which are comparable to fingerprints. By comparing these bio-optical signatures to an on-board database of microorganism patterns, detection and classification occurs within minutes. The system is cost effective, uses no reagents, operates remotely, and can be used for continuous microbial surveillance in many water treatment environments. Tests with BioSentry in a distribution network pilot showed that turbidity significantly affected baseline calibration [54].

Table 3.3. Commercial devices for detection of microorganisms in water

Name	Company	Technology	Paremetres	Analysis time	LOD	Cost	Characteristics
SECTOR® Imager 2400	Meso-Scale Discoveries (USA)	Electrochemi-luminescence	N/A	3 min for plate count	10 000 -100 000 CFU/100 ml	N/A	Lab device. Automated multiplexed ECL assays in array format. Carbon ink electrodes in multiwell plates with CCD camera
GeneXpert®	Cepheid (USA)	PCR	Depends on the probe	20min – 2h	100-10 000 CFU/100 ml	$30000	Lab Device. Very focused to clinical use. GeneXpert automates the process but would require an automatic sampler
AquaScope	AquaExplorer (Netherlands)	FISH and filter cytometry	*E. coli, enterococcus, legionella* (several probes available)	20min - 2 h	100 CFU/100 ml	€85000	Lab device. Adaptable to operate on-line
Rheonix CARD™	Rheonix (USA)	PCR	*E. coli* and *Enterococci*	3-4 h	100 CFU/100 ml	$7500	Portable. Manual filtration of sample. Up to 4 simultaneous assays
Profile-1®	NewHorizons (USA)	ATP bio-luminescence	Overall bacterial ATP	5-30 min + manual sample preparation	100 000 -100 000 CFU/100 ml	$4000	Portable. Manual reagent dosage
P-CAN™ Sensor	IBI (USA)	B cell expressing bioluminescent protein and antibody	*E. coli* + others	5 min + manual sample preparation	5 000-10 000 CFU/100 ml	$15000	Portable. Centrifugal rotor promotes binding. Luminescence read in real time with photomultiplier tube
RAPTOR™	Research International (USA)	Fibre-Optic fluoro-immunoassay	*E. coli* + others	10-15 min + manual sample preparation	100 000 CFU/100 ml	$49000	Portable. Manual sample load monitoring of fluorescently-labeled chemical reactions. Graphical display of data recovery
Enigma-FL	Enigma Diagnostics (UK)	PCR	*E.coli* + others (depends on the probe)	30-40 min	1 000-100 000 CFU/100 ml	$70000	Portable. Sample is inserted in cartridges. Automated sample processing integrated with PCR fluorescent detection
AquaBio	ADASA (Spain)	Enzyme fluorescence	*E. coli* + total coliforms	3-18h	1 CFU/100 ml	N/A	Online. Based on Colilert-18 reagents by IDEXX
Calm	COLIFAST (Norway)	Enzyme fluorescence	*E.coli,* total coliforms, thermotolerant coliforms, *P. aeruginosa*	2-12h (early warning in 4h)	1 CFU/100 ml	N/A	Online. Fully automated. Tests may be performed simultaneously
Alarm	COLIFAST (Norway	Enzyme fluorescence	*E.coli*, total coliforms, thermotolerant coliforms	6-14h	1 CFU/100 ml	N/A	Online. Same Analysis technology than Calm device, but industrial grade. Also measures turbidity

Table 3.3. Commercial devices for detection of microorganisms in water (cont.)

Name	Company	Technology	Paremetres	Analysis time	LOD	Cost	Characteristics
Kolisoon	ISRIM (Italy)	Enzyme fluorescence	*E.coli*	1-10h	10 CFU/100 ml	N/A	Online. Includes autosampler, automatic filtration, reagents dosing station, reaction carousel and fluorimeter
Coliguard	mbOnline (Austria)	Enzyme fluorescence	*E. coli*	30min-4h	0.3 CFU/100 ml	€45000	Online. Fully automatic. Sample volume ranging 20-5000ml
Spyglass™ ESP System	Spyglass Biosciences (USA)	DNA probe arrays (qPCR)	Bacteroides	N/A	N/A	N/A	Online. Submerged on water. Fully autonomous platform from sample collection to result transmission to the end user
Luminos Lab-on-Line	Lab Bell (Canada)	Enzymatic activity kinetic (M.E.R)	*E. coli* and *enterococci*	20min -3h	100 CFU/100 ml (enterococci) 400 CFU/100 ml (coliforms)	€7300 (portable) €28000 (on-line)	1 sample per run. The conversion between the fluorescence and the level of contamination must be calibrated for each sample type
Predect™	Predect (Sweden)	Multi Angle Light Scattering (MALS)	None. Detects particle shape	Continuous	N/A	N/A	On-line. Light scattering patterns analysed by software algorithm
BioSentry™	JMAR (USA)	Multi Angle Light Scattering (MALS)	None. Detects particle shape	Continuous	60 000 CFU/100 ml	$47000	On-line. Compares light scattering patterns to "Bio-Optical signatures" and classifies them
Pathogen Sensor	Early Warning Inc.(USA)	Multiplexing DNA electrochemical biosensor	*E.coli* and *Salmonella* (other probes available)	3h	1 CFU/100 ml (bacteria) 10 cells/10L (protozoa) 10 part/10L (virus)	N/A	Fully autonomous platform from sample collection (10L) to result transmission to the end user (SCADA interface)
AWISS	Environmental Technology Solutions, LLC (USA)	Enzyme fluorescence	*E. coli*	1-8h	100 CFU/100 ml	N/A	Battery-powered device, which contains a prototype optical sensor that can measure changes in fluorescence intensity

Evaluation of sensors for real-time monitoring of *E. coli* in water distribution systems was performed by Miles [55]. Only BioSentry was fairly accurate; however, the sensor could not distinguish between particulates and *E. coli* if concentrations were relatively high. The response of the TOC sensors to intrusion events was variable, being more sensitive in the detection of the media associated with *E. coli* than the microorganisms themselves.

AWISS, an autonomous wireless in-situ sensor, was used for the detection of *E. coli* [56]. A prototype battery-powered optical AWISS, consisting of a miniature spectrophotometer, monitored the changes in fluorescence intensity that occurs when the *E. coli*–synthesised b-glucuronidase enzyme hydrolyses the reagent's glycosidic bond, releasing fluorophores into solution. Laboratory testing with the prototype sensor showed the AWISS was capable of detecting low concentrations (< 100 CFU/100 mL) in less than eight hours. Higher concentrations (> 5 000 CFU/100mL), indicative of a possible combined sewage discharge, could be detected in less than one hour.

After performing the review it could be stated that on-line systems should include sample preparation (at least a concentration of contaminants) and detection of the analytes. Consequently they will be somewhat complex to develop and use. On-line concentration is a challenge by itself (although it has not been specifically addressed in this review).

The next challenges for the development of on-line systems will be to meet the following requirements: acceptable specificity and sensitivity, acceptable cost and acceptable maintenance. Further support for their viability is a need to interpret data in real time and implement a management strategy in response.

3.5. Conclusions

We have identified and reviewed technologies for water quality analysis of faecal contamination indicators. Some established technologies used in microbiology laboratories, such as immunoassays or enzyme fluorescence, have the potential to be applied in on-line equipment if combined with automated detection methods and implementing proper enhancements for reduction of the analysis time. More recent technologies such as PCR, FISH or electrochemiluminescence enable the quantification of very low concentrations of bacteria with high specificity, but require a lot of sample processing and have not been applied for on-line raw samples. Optical techniques provide the advantage of being immediate and reagentless, so that combined with capture methods may become the ideal detection technology for online water monitoring. However, to date it has been difficult to obtain successful results in real samples in a simple and reliable manner.

We performed an identification of devices based on the previous technologies, mainly aimed at generating an alarm when there is an episode of accidental or deliberate faecal contamination. Detection of microorganisms either in very low concentrations or within a turbid complex matrix is a challenging task, especially when aiming for online monitoring. All in all, although significant advances have been made in recent years in technologies – and its applications – to monitor water, it can be envisaged that while many of them seem promising, they are not currently available and require further testing and/or development.

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4

Advanced monitoring of pharmaceuticals and oestrogens in the Llobregat River basin (Spain) by liquid chromatography-triple quadrupole-tandem mass spectrometry in combination with ultra-performance liquid chromatography time-of-flight mass spectrometry

4. Advanced monitoring of pharmaceuticals and oestrogens in the Llobregat River basin (Spain) by liquid chromatography-triple quadrupole-tandem mass spectrometry in combination with ultra-performance liquid chromatography time-of-flight mass spectrometry

4.1. Abstract

We investigated the occurrence of 28 pharmaceuticals and 10 oestrogens in waters from the lower part of the Llobregat River basin, where the main intakes for production of drinking water for Barcelona (Spain) are located. Sampling was scheduled to monitor the same mass of water on its way down the river to reflect inputs from discharges, the contribution from subsidiaries plus the persistence of the compounds in the surface water. Analysis of pharmaceuticals was performed by off-line solid phase extraction (SPE) followed by liquid chromatography tandem mass spectrometry with a triple quadrupole analyser (LC-QqQ-MS/MS). Further analysis by ultra-performance liquid chromatography/mass spectrometry with a time-of-flight analyser (UPLC-TOF-MS) was proposed and applied for confirmation of several of these target compounds. Oestrogens were analysed by on-line SPE-LC-QqQ-MS/MS. Within the class of pharmaceuticals, 23 out of the 28 compounds investigated were detected in at least one sample. The highest concentrations were observed for the β-blockers metoprolol (8042 ng L^{-1}) and sotalol (788 ng L^{-1}), the antibiotic ofloxacin (1904 ng L^{-1}), and the lipid regulator gemfibrozil (1014 ng L^{-1}). Within the group of oestrogens, only oestrone and oestrone-3-sulphate were positively identified, with concentrations for the former (0.82 to 5.81 ng L^{-1}) close in some locations to those considered sufficient to induce oestrogenic effects in aquatic organisms (1-10 ng L^{-1}). As a general pattern, the concentration of target compounds increases along the river flow as expected.

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4.2. Introduction

This study is focused on monitoring the presence of pharmaceutically active compounds in natural waters. This family of compounds includes prescription drugs, over-the-counter medications and drugs used in hospitals, plus other natural hormones having endocrine disrupting properties.

Interest about the study of the presence and toxicity of these compounds has been reported by some international organisations (GWRC, 2004)(Henderson, 2006). The European parliament, for instance, during the preparation of the recently adopted Directive 2008/105/EC on environmental quality standards in the field of water policy, considered the inclusion of various pharmaceuticals (e.g. carbamazepine and diclofenac) inthe list of substances subject to a review for possible identification as "priority substances" or "priority hazardous substances", although they were finally withdrawn from the final version. The Global Water Research Coalition in an effort to develop a common list of pharmaceuticals relevant to the water cycle has included carbamazepine, sulfamethoxazole, diclofenac, ibuprofen, naproxen, bezafibrate, atenolol, erythromycin and gemfibrozil as Class1: high priority pharmaceuticals (GWRC, 2004).

The main reasons for concern are that large quantities of these compounds can enter the environment after use by individuals. In recent years, several studies have shown the efficiency of water treatment technologies in removing pharmaceutical and endocrine disrupting compounds. Whereas conventional technologies such as sand filtration or flocculation showed poor elimination percentages (Ternes et al., 2002), advanced oxidation processes (Ternes et al., 2003; Zwiener and Frimmel, 2000) or nanofiltration and reverse osmosis techniques (Snyder et al., 2007) have been shown to be the most effective ones. Concerning secondary treatments in waste water treatment plants (WWTPs), the literature shows activated sludge with nitrogen treatment and membrane bioreactor as the most efficient treatments (Miège et al., 2009). Other sources are unused or expired medications that are thrown away in rubbish, residues from pharmaceutical manufacturing and waste from hospitals (www.epa.gov/ppcp).

Recent advances in technology have improved the ability to detect and quantify these chemicals in environmental samples. Even though they are found in very low concentrations, there is still a lack of knowledge about the long-term risks that the presence of a large variety of drugs may pose for non-target organisms as well as for human health (Gros et al., 2006)

The lower Llobregat River basin (NE of Spain) has been the object of several studies dealing with the presence of these target analytes in surface water. A high concentration of industrial and agricultural activities, added to the fact that is a densely populated area, make these waters receiving bodies for urban and industrial WWTPs, accidental spills from industries and general pollution from agriculture. This situation, due to the fact that the Llobregat River is the source of drinking water for millions of

inhabitants living in the area, raises the necessity of further investigation into the presence of these pollutants and the associated risks.

Pharmaceuticals and their toxicity have been studied in the upper part of the Llobregat basin (Farré et al., 2001) and a short list of them were included in a study covering a wide range of emerging pollutants in the same area (Kuster et al., 2008). Results obtained in the Llobregat basin area, plus other campaigns in other basins in Spain (Gros et al., 2006; Hernando et al., 2006), show levels of pharmaceuticals at the nanogram-per-litre level (ng L^{-1}) or even at the low microgram-per-litre level (µg L^{-1}). Oestrogens and progestogens have also been monitored in this area (Petrovic et al., 2002; Rodriguez-Mozaz et al., 2004a; Rodriguez-Mozaz et al., 2004b; Solé et al., 2000) showing levels of some of them, especially oestrone and oestrone-3-sulphate, at the low nanogram-per-litre level (ng L^{-1}).

For the analysis of these compounds in the studies reviewed, liquid chromatography/mass spectrometry (LC-MS) has been the technique mainly selected in the past (Farré et al., 2001; López De Alda and Barceló, 2000), but now this technique has been largely replaced by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Gros et al., 2006; Hernando et al., 2006; Kuster et al., 2008). In recent years, the Commission Decision 2002/657/EC that was aimed at regulating the performance of analytical methods in the food industry, has also been applied to environmental analysis. According to this regulation, three identification points (IP) are needed when using LC-MS/MS for the correct confirmation of the presence of target compounds. The high sensitivity of LC-MS/MS (with triple quadrupole (QqQ) analysers) makes it a very suitable, accessible technique for analysis in surface waters. The main problem is that the three IPs are not obtained for those analytes not showing two selected reaction monitoring (SRM) transitions, that is, when only one product ion can be obtained from the precursor one. This disadvantage makes this technique not reliable enough for the analysis of compounds such as ibuprofen or gemfibrozil.

An innovative aspect of this study is the performance of an extra analysis based on the use of time-of-flight (TOF) detection for confirmation of the analytes, an approach that has been previously tested for analysis of pharmaceuticals but in wastewaters (Martínez Bueno et al., 2007). Ultra-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry (UPLC-QTOF-MS/MS) has been applied for drugs identification in wastewater analysis (Petrovic et al., 2006). However, the use of this technique was tested in this study and finally discarded for our purposes because of the high detection limits found (results not included in this study). UPLC-TOF-MS was also tried and finally selected because of its higher sensitivity. Detection limits were appropriate to confirm peaks of contamination of target analytes in surface waters. TOF-MS measures the accurate mass of the compounds, adding that extra point of confirmation needed in order to obtain a reliable result.

Another innovative aspect concerns the sampling method. Instead of taking samples from several points at the same time, sampling was scheduled to try to sample the same mass of water on its way down the river. Therefore, monitoring should reflect inputs from discharges, the contribution from subsidiaries plus the persistence of the compounds in the surface water. This sampling method helps to eliminate some sources of mistakes when interpreting results obtained from traditional samplings (e.g. a peak of contamination caused by a punctual discharge upstream will not be detected in the analysis downstream and this could lead to a wrong conclusion regarding the natural removal of the compound).

This work also provides, for the first time, a view on the occurrence of 38 emerging compounds in the lower part of the Llobregat basin area based on the combination of two techniques (LC-QqQ-MS/MS + UPLC-TOF-MS) for their unequivocal confirmation and quantification.

4.3. Material and methods

4.3.1. Chemicals and standards

All standards used were of high purity grade (>90%). Ibuprofen, naproxen, ketoprofen, diclofenac and gemfibrozil were kindly supplied by Jescuder (Rubí, Spain). Indomethacine, acetaminophen, mefenamic acid, clofibric acid, bezafibrate, mevastatin, azythromycin dihydrate, erythromycin hydrate, carbamazepine, fluoxetine hydrochloride, lansoprazole, loratadine, famotidine, ranitidine hydrochloride, sulfamethoxazole, trimethoprim, ofloxacin, atenolol, metoprolol, propanolol hydrochloride and sotalol hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Propyphenazone, pravastatin and paroxetine hydrochloride were from LGC Promochem (London, UK). Pure standards of the natural and synthetic, both free and conjugated, oestrogens oestriol-3-sulphate, oestriol-16-glucuronide, estradiol-17-glucuronide, oestrone-3-glucuronide, oestrone-3-sulphate, oestriol, estradiol, ethynyl estradiol, oestrone and diethylstilbestrol were supplied by Sigma Aldrich (Steinheim, Germany)

Individual stock standard solutions were prepared at 1000 µg mL^{-1} in methanol and stored at −20 ˚C. A mixture of all pharmaceutical standards and another mixture containing all oestrogens were prepared by the appropriate dilution of the individual stock solutions. Further dilutions of the pharmaceutical mixture were prepared in methanol–water (25:75, v/v) before each analytical sequence and were used as working standard solutions for external calibration. Working standard mixtures of the oestrogens were prepared by dilution in methanol and used as spiking solutions for preparation of the aqueous calibration standards (content of methanol < 0.1%).

HPLC-grade acetonitrile and water (Riedel de Haën) were supplied by Sigma Aldrich (Steinheim, Germany). Methanol (J.T. Baker) was supplied by Serviquimia (Constantí, Spain). Hydrochloric acid 37%, ammonium acetate (NH_4Ac) and acetic acid (HAc) were from Merck (Darmstadt, Germany). Nitrogen for drying 99.995% of purity was from Air Liquide (Madrid, Spain).

4.3.2. Site description and sampling procedure

The Llobregat River is located in the northeast of Spain and flows into the Mediterranean Sea south of the city of Barcelona. This is a densely populated area where agriculture and industrial activities (tannery, textile, pulp, paper and salt mining) are also present. The river receives discharges from urban and industrial WWTPs and runoff from agriculture and salt formation areas. Water from the Llobregat River basin is also used for the production of drinking water. Several drinking water plants are located next to the river. This high urbanisation of the basin is especially significant in Mediterranean climate basins. River water flows fluctuate heavily throughout the year and wastewater effluents can account for the majority of the river water flows during the dry season. The river represents, together with its two main tributaries, the Cardener River and the Anoia River, a good example of overexploited Mediterranean rivers.

In this study we collected a total of 16 water samples at eight selected sites in the lower reach of the Llobregat River basin (see Figure 4.1) in two different sampling campaigns performed in November 2006 and December 2006. The first site was located upstream just before the Terrassa Drinking Water Treatment Plant (DWTP) intake (site 1). The five following samples were taken at sites (2 to 6) located downstream of the Llobregat basin. According to the flow, an estimation of the time to take the samples was performed with the aim of monitoring the same water mass as it flows to the sea. Site 2 was located in the Llobregat River just before the union with its tributary, the Anoia River, which carries the discharge of the Abrera WWTP. The next site was located in the Anoia River itself close to its confluence with the Llobregat River (site 3). The following samples were taken from the Llobregat River at Capdevila Dam (site 4, before the input of the Rubí Creek), downstream of the town Molins de Rei (site 5), and before the intake of the Sant Joan Despí DWTP (site 6), the biggest DWTP supplying water to the city of Barcelona. Additionally, we monitored two more sites (A and B) having less influence in the water quality of site 6: a channel receiving polluted water from the Anoia River, the Rubí Creek and the Sant Feliu WWTP (site A) that was constructed to avoid these waters being discharged into the Llobregat River before the Sant Joan Despí DWTP intake (site A), and the Rubí Creek itself (site B) which receives wastewater from industries in the area.

Water samples (2 L) were collected in amber glass bottles to avoid photodegradation of the analytes. Upon reception, samples were filtered through 0.45 μm Nylon filters (Whatman, Maidstone, UK) to

eliminate particulate matter and other suspended solid matter and then stored at 4 ºC in the dark until analysis which was always carried out within 48 h of collection to keep microbial degradation to a minimum.

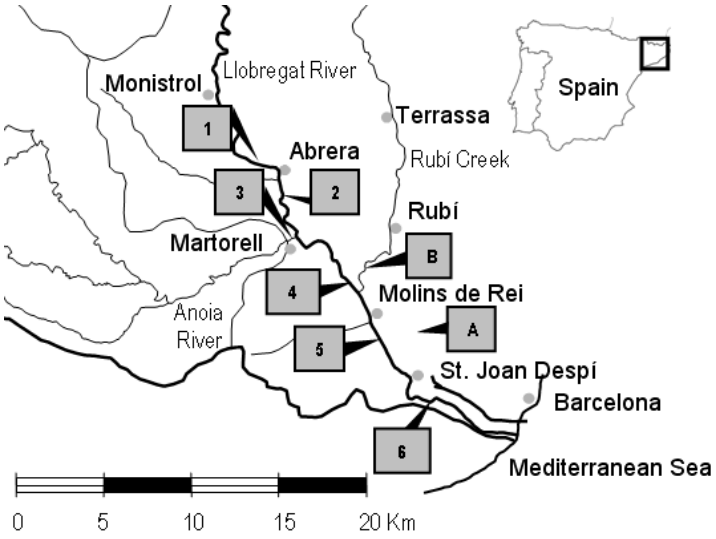

Figure 4.1. Map of the lower Llobregat basin where the eight sampling sites are indicated (1-6, A, B)

4.3.3. Analytical methods

Pharmaceuticals were initially analysed by off-line solid phase extraction (SPE) followed by LC-QqQ-MS/MS. Additional confirmation was performed by UPLC-TOF-MS. Oestrogens were monitored using an on-line SPE-LC-QqQ-MS/MS method.

4.3.3.1. Determination of pharmaceuticals by off-line SPE followed by LC-QqQ-MS/MS

Pharmaceuticals were initially analysed by off-line SPE followed by LC-QqQ-MS/MS according with a method described in the literature (Gros et al., 2006).

MS/MS detection was performed in the SRM mode, obtaining two SRM transitions per compound. Only 1 SRM transition was obtained in the case of ibuprofen, gemfibrozil, and pravastatin due to poor fragmentation. A second transition was obtained for ofloxacin and ketoprofen according to the method followed, but it could be not detected in the experiment. In order to increase the sensitivity, the SRM transitions were classified into different elution time windows. The first transition, the most abundant one, was used for quantification, and the second transition, the less abundant, was used for confirmation purposes.

Identification of the target analytes was achieved by comparing the retention time and the LC-MS/MS signals of the target compounds in the samples with those of standards analysed under the same conditions. For positive identification the following criteria had to be met: (1) LC chromatographic retention time agreement within 2%; (2) relative abundance of the two selected precursor ion-product ion transitions within a margin of 20% (93/256/EEC).

4.3.3.2. Determination of oestrogens by on-line SPE- LC-QqQ-MS/MS

Fully automated on-line SPE-LC-QqQ-MS/MS analysis of oestrogens was performed with a SPE sample processor Prospekt-2 (Spark Holland, Emmen, The Netherlands) coupled on-line to the LC-MS/MS system. The method was previously developed for the analysis of free oestrogens (Rodriguez-Mozaz et al., 2004a) but it has been modified to include three conjugated compounds (oestrone-3-glucuronide, oestriol-3-sulphate and oestriol-16-glucuronide).

MS/MS detection was performed in the SRM mode with an electrospray interface operated in the negative ion (NI) mode. 2 SRMs transitions were monitored per compound. For quantification of the analytes, the external standard method was used, based on the peak areas obtained in the first SRM transitions.Positive confirmation of the target analytes in the samples was based on the same criteria (retention time and relative abundance of the 2 SRM transitions signals) described in section 2.3.1.

4.3.3.3. Confirmation of pharmaceuticals by UPLC-TOF-MS

Confirmation of pharmaceuticals in the water sample extracts previously analysed by LC-QqQ-MS/MS was performed by UPLC-TOF-MS. The method is a modification of a previously developed UPLC-QTOF-MS/MS method (Petrovic et al., 2006).Positive identification of the target compounds was based on: (a) accurate mass measurement of the analyte base peak with an error <5 ppm; and (b) LC retention time of the analyte compared to that of a standard within ±2%.

In all cases (the only exception was pravastatine, which formed the sodium adduct) the base peak corresponded to the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ion of the analyte, depending on whether the analysis was performed in the positive ion (PI) or NI mode. The errors obtained in mass measurements (between 0.0 and 4.7 ppm (0.0–1.2 mDa) were within the widely accepted accuracy threshold of 5 ppm.

For low contaminated waters (river, ground and drinking water) the instrumental detection limits (IDLs) might not be sufficient to detect low concentrations occurring in these samples, and the UPLC-TOF-MS

method needs to be complemented by a sensitive quantitative analysis using a QqQ in SRM mode (Petrovic et al., 2006). That is why only analytes showing one transition or achieving the highest levels in the QqQ analysis were selected for confirmation, as QqQ analyses have been shown to be more sensitive and accurate for quantification. Therefore, confirmation analysis via UPLC-TOF-MS was done only for diclofenac, ibuprofen, gemfibrozil, atenolol, sotalol, metoprolol and ofloxacin in the most polluted samples (sites 3, 5, A, B).

4.4. Results and discussions

4.4.1. Levels of Pharmaceuticals

Table 4.1 lists the method detection and quantification limits calculated for the quantification and confirmation SRM transitions monitored for the various target pharmaceuticals in the off-line SPE-LC-QqQ-MS/MS method, together with the percentage of positive samples and the minimum, maximum and average concentrations quantified using this method in the samples investigated.

For all pharmaceuticals except ibuprofen, gemfibrozil, pravastatin, ketoprofen and ofloxacin, 2 SRM transitions were detected per compound thus achieving 4 IPs (2002/657/EC), which is in compliance with the minimum confirmation requirements established in the Council Directive 96/23/EC. However, in the case of ibuprofen, gemfibrozil, pravastatin, ketoprofen and ofloxacin, for which only 1 SRM transition was detected or monitored due to poor fragmentation in the MS/MS system, only 2.5 IPs were obtained, which are not enough to comply with the aforementioned Directive.

In order to gain sufficient confirmation in the analysis of these five compounds, water sample extracts previously analysed by LC-QqQ-MS/MS were subjected to a second analysis by means of UPLC-TOF-MS, which obtained 2 additional IPs per ion monitored (2002/657/EC). As shown in Table 4.2, ibuprofen and gemfibrozil could be positively confirmed in the samples through the second analysis (LODs 150 and 50 ngL^{-1}, respectively). Pravastatin and ofloxacin levels, in spite of reaching 78 and 1904 ngL^{-1}, respectively, in the samples, were too low for confirmation using this second technique (LODs 350 and 500 ngL^{-1}, respectively). Ketoprofen had not been found by means of LC-QqQ-MS/MS so no confirmation was performed by UPLC-TOF-MS (LOD 150 ngL^{-1}).

Table 4.1. Method detection and quantification limits for the various target pharmaceuticals in the off-line SPE-LC-QqQ-MS/MS method, together with the percentage of positive samples and the minimum, maximum and average concentrations quantified using this method in the samples investigated

Therapeutic group	Compound	LOD (ng/L) SRM 1	LOQ (ng/L) SRM 1	LOD (ng/L) SRM 2	%Rec.	% Positive Samples	Max	Min	Average	Max[a]	Average[a]
Analgesics and anti-inflammatories	Ketoprofen	0.6	1.3	-	54-115	0	-	-	-	144	10.8
	Naproxen	0.3	0.7	0.5		100	105.5	14.4	42.6	4500	14.8
	Ibuprofen	0.5	1.4	-		100	490.4	29.4	152.9	6400	101.1
	Indomethacin	0.6	1.6	3.0		50	46.7	10.1	23.8	220	3.5
	Diclofenac	0.4	0.9	3.1		100	358.1	17.6	87.7	1800	19.6
	Mefenamic acid	0.1	0.2	0.7		14	2.4	2.4	2.4	242	16.0
	Acetaminophen	0.1	0.4	0.9		100	96.5	14.1	34.8	3600	30.9
	Propyphenazon	0.1	0.4	0.4		100	18.8	1.5	7.3	880	25.0
Lipid regulators and cholesterol lowering statin drugs	Clofibric acid	0.1	0.1	0.6	101-116	21	13.3	2.1	7.0	630	16.9
	Gemfibrozil	0.1	0.3	-		100	1014.1	26.1	242.8	58000	15.9
	Bezafibrate	0.1	0.2	0.5		100	305.2	3.4	48.3	780	12.5
	Pravastatin[b]	4.2	11.1	-		21	77.7	52.6	65.1	0	0.0
	Mevastatin	0.1	0.1	0.1		0	-	-	-	0	0.0
Antiulcer agent	Lansoprazole	0.6	1.5	2.5	111	14	76.7	42.0	59.3	0	0.0
Histamine H1 and H2 receptor antagonists	Loratadine	0.2	0.4	0.4	38-70	57	201.6	0.9	45.5	-	-
	Famotidine	0.3	0.7	0.7		21	8.6	3.5	6.4	-	-
	Ranitidine	0.4	0.9	0.3		100	69.6	2.3	16.5	142	10.0
Antibiotics	Erythromycin	0.9	2.3	1.6	52-113	93	111.9	6.9	32.9	1209000	52.3
	Sulfamethoxazole	0.3	0.9	0.4		100	119.3	4.1	24.0	1900	35.6
	Trimethoprim	0.2	0.5	0.3		100	252.0	2.4	38.5	800	18.2
	Ofloxacin[b]	0.9	2.3	-		100	1903.6	8.0	285.3	306	40.6
ß-Blockers	Atenolol	0.1	0.4	0.2	58-102	100	199.7	5.8	41.5	465	106.3
	Sotalol	0.1	0.3	0.2		100	787.6	1.9	66.8	950	49.3
	Metoprolol	0.3	0.7	0.4		79	8041.1	1.2	738.0	2200	47.4
	Propranolol	1.1	3.0	0.5		64	17.3	1.6	6.7	590	8.0
Psychiatric drugs	Carbamazepine	0.1	0.1	0.2	83-95	100	178.7	8.3	64.0	2500	68.9
	Fluoxetine	1.4	3.8	0.4		7	4.2	4.2	4.2	34	10.5
	Paroxetine	0.4	1.0	1.3		0	-	-	-	0	0.0

[a] Concentration in surface water according to http://www.knappe-eu.org/fichiers/44-D1.2 environmental indicator final version.pdf

[b] Not confirmed by UPLC-TOF-MS

This approach was also used to confirm the presence of compounds detected at very high concentrations in heavily polluted samples. Since the sensitivity provided by LC-QqQ-MS/MS working in the SRM mode is higher than that achieved by UPLC-TOF-MS in the scan mode, not all positive results obtained using the former technique can be confirmed by using the latter. However, for those cases where the concentrations found are very high, an additional source of confirmation is possible and valuable. Table 4.2 shows as an example the results obtained in the UPLC-TOF-MS analysis of diclofenac, atenolol, sotalol and metoprolol in samples collected from sites 3, A and B. In this case, all the results were positively confirmed.

Regarding the results obtained, 14 out of the 28 pharmaceuticals investigated were detected in all samples (see Table 4.1). Only three compounds, namely, ketoprofen, mevastatin, and paroxetine were not detected in any sample. Figure 4.2 shows the range of concentrations measured for the various compounds positively identified in the samples. The highest concentrations, above 500 ngL^{-1}, were found for the lipid regulator gemfibrozil (up to 1014 ngL^{-1}), the antibiotic ofloxacin (up to 1904 ngL^{-1}), and the β-blockers sotalol (up to 788 ngL^{-1}) and metoprolol (up to 8042 ngL^{-1}). Average concentrations higher than 100 ngL^{-1} were calculated for the analgesic anti-inflammatory ibuprofen (153 ngL^{-1}), and also for gemfibrozil (243 ng ngL^{-1}), ofloxacin (285 ngL^{-1}) and metoprolol (738 ngL^{-1}) (Table 4.1). Carbamazepine was among the most ubiquitous compounds (detected in all samples). Concentrations ranged from 8 to 179 ng ngL^{-1}. Although its consumption is not very high, carbamazepine is not or is very poorly removed in conventional treatment processes operating in WWTPs (Zhang et al., 2008).

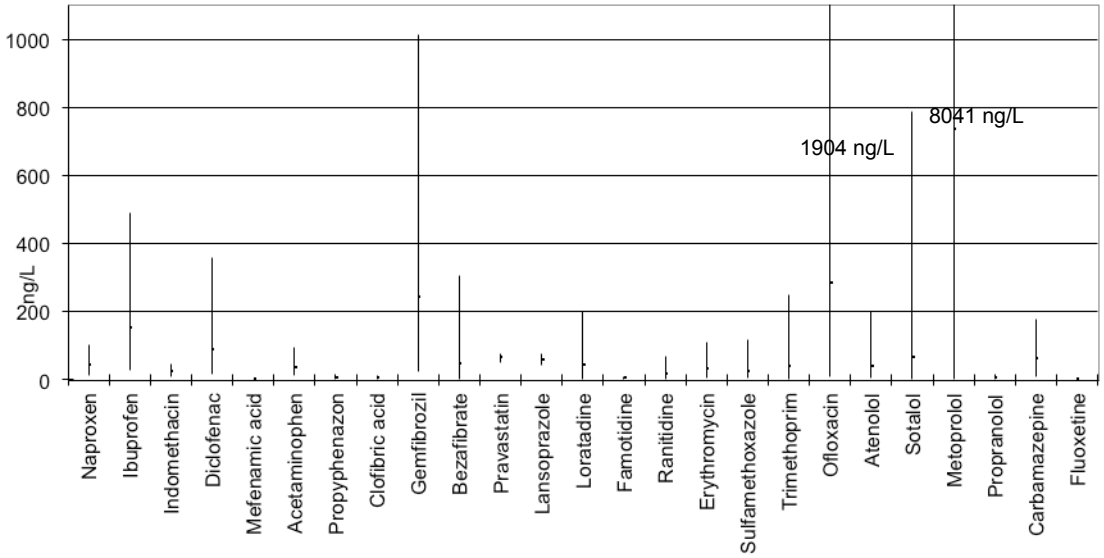

Figure 4.2.Range of concentrations (minimum, maximum and average) measured for the various compounds positively identified in the samples (ng L^{-1})

Table 4.2. Examples of accurate mass measurement of selected pharmaceuticals in real samples at selected sites

		Site B November 2006			Site A November 2006			Site 3 November 2006			Site B December 2006		
Compound	Theoretical mass (m/Z)	Experimental mass (m/Z)	Error mDa	ppm	Experimental mass (m/Z)	Error mDa	ppm	Experimental mass (m/Z)	Error mDa	ppm	Experimental mass (m/Z)	Error mDa	ppm
Diclofenac	294.0089	294.0083	-0.2	-0.6	294.0890	0	0				294.0076	-1.3	-4.4
Ibuprofen	205.1229	205.1229	0.0	0.0	205.1238	0.9	4.4	205.123	0.1	0.5			
Gemfibrozil	249.1491	249.1489	-0.2	-0.8	249.1483	-0.8	-3.2				249.1487	-0.4	-1.6
Atenolol	267.1708	267.1704	-0.5	-1.9									
Sotalol	273.1273				273.1263	-1.0	-3.7						
Metoprolol	268.1912				268.1921	0.8	0.3						
Ofloxacin	362.1516	-	-	-	-	-	-				-	-	-

Recently, an extensive data compilation on the environmental occurrence of pharmaceuticals products in surface water and WWTP influents and effluents has been performed in the context of the project KNAPPE - Knowledge and Need Assessment on Pharmaceutical Products in Environmental Waters funded by the European Commission within the 6th Framework Programme (Sadezky et al., 2008). Comparing the average concentration calculated for each compound within this study with the average of those compiled in the context of the above project, all detected pharmaceuticals except mefenamic acid, propyphenazon, clofibric acid, erythromycin, sulfamethoxazole, atenolol, propanolol, carbamazepine, and fluoxetine, (i.e. 16 compounds in total), showed higher values. Much higher values were those relating to gemfibrozil (243 ngL^{-1} *vs* 16 ngL^{-1}), ofloxacin (285 ngL^{-1} *vs* 41 ngL^{-1}), and metoprolol (738 ngL^{-1} *vs* 47 ngL^{-1}). However, the maximum values reported were only exceeded by ofloxacin (1904 ngL^{-1}*vs* 306 ngL^{-1} found in Italy) and metoprolol (8041 ngL^{-1} *vs* 2200 ngL^{-1} found in Germany), in addition to those never detected in the reviewed literature, namely, pravastatin (maximum concentration in this work 78 ngL^{-1}), lansoprazole (77 ngL^{-1}), loratadine (202 ngL^{-1}), and famotidine (9 ngL^{-1}).

Classified by therapeutic groups, the highest average concentrations were detected for the β-blockers (average of positive results = 191 ngL^{-1}), followed by lipid regulators and cholesterol lowering statin drugs (118 ngL^{-1}), antibiotics (91 ngL^{-1}), analgesics anti-inflammatories and antiulcer agents (both 59 ngL^{-1}), psychiatric drugs (58 ngL^{-1}), and histamine H1 and H2 receptors antagonists (23 ngL^{-1}). For the discussion of results it is important to mention that samples from site 2 and site A taken in the second sampling period were lost during sample preparation.

Figure 4.3 shows the total charge of pharmaceuticals, grouped by therapeutic class, detected along the course of the river basin, at both sampling campaigns. Sites showing the highest concentrations of the target compounds were site A (channel receiving waters from the Anoia River, Rubí Creek and Sant Feliu WWTP) and site B (Rubí Creek). A decision was taken in the past to avoid these waters discharging to the Llobregat River upstream of the Sant Joan Despí DWTP.

Monitoring of surface water along the river flow showed, in general, an increase of the total pharmaceuticals concentrations from site 1 to 6. The exception was site 3, which is not located in the Llobregat River itself but in the tributary the Anoia River before it joins the Llobregat River. Levels of target compounds were usually higher in the Anoia River (site 3). The low flow of the Llobregat River, linked to its seasonal fluctuations, makes this river very sensitive to the levels of target analytes present in its tributaries and the water coming directly from WWTPs. Comparing both campaigns, slightly higher values were found in the samples collected in December. Taking into account the proximity of sampling periods, results indicate a fairly constant input of contaminants into the river basin.

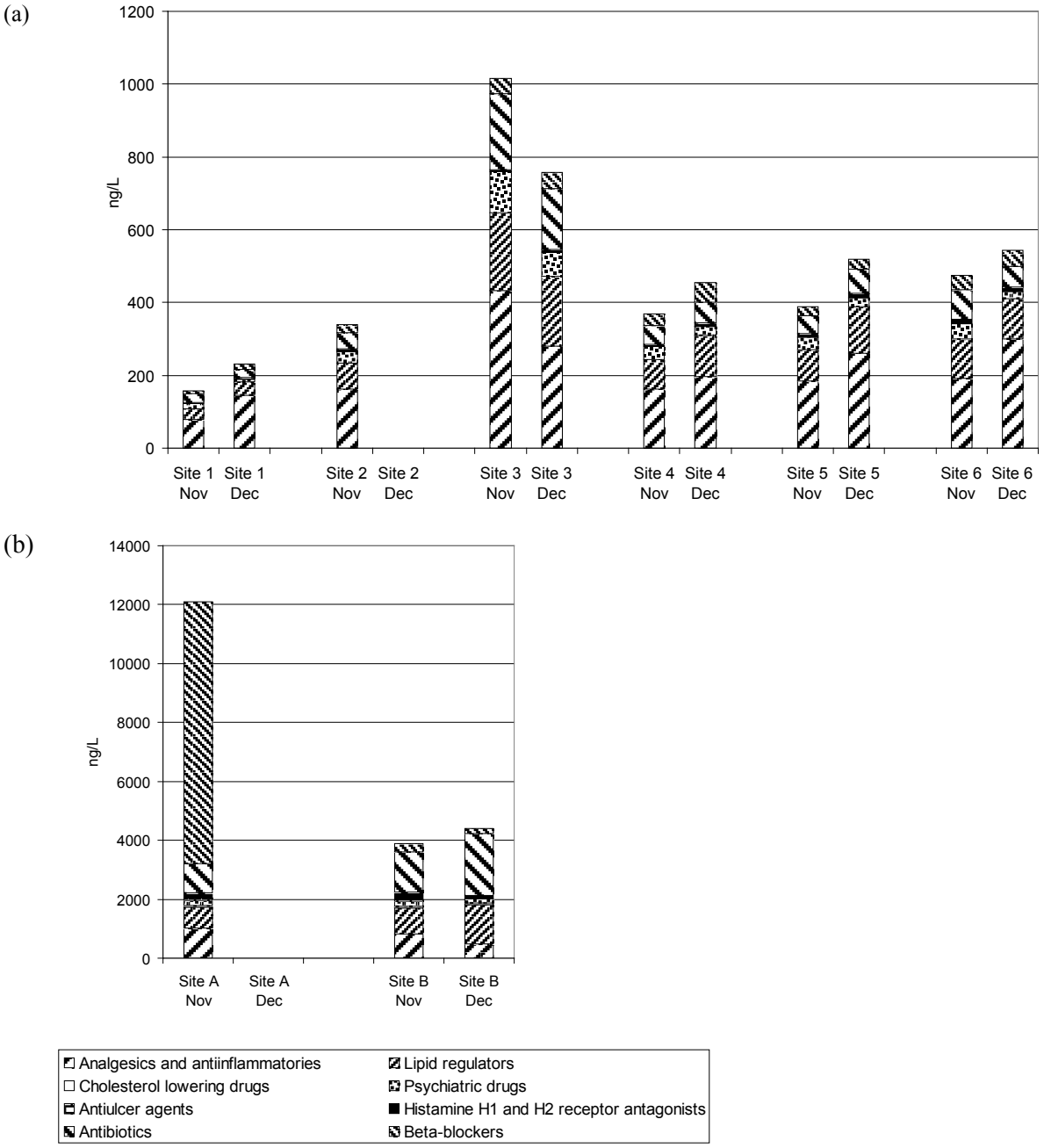

Figure 4.3. Cumulative levels of pharmaceuticals, grouped by therapeutic class, detected at sites 1-6 (a) and sites A and B (b), at both sampling campaigns

Information on acute and, especially chronic toxicity of pharmaceutical aquatic waste is scarce. Pharmaceutical concentrations measured in surface waters are generally well below concentrations that are known to cause acute toxicity to aquatic organisms (Cooper et al., 2008). However, pharmaceuticals enter the aquatic environment continuously, leading to fairly constant environmental water concentrations. Chronic exposure to pharmaceuticals has the potential for numerous subtle effects, such as metabolic or reproductive changes on non-target organisms (Cooper et al., 2008).The UK Environment Agency (Boucard, 2006) has recently compiled a database including data on the chronic aquatic

ecotoxicity of human pharmaceuticals towards various aquatic organisms belonging to different taxonomic groups. The maximum concentrations measured in the Llobregat basin were always below the reported chronic toxicity values. Maximum concentrations measured were on average more than 5, 3, 6, and 5 orders of magnitude lower than the lowest toxicity values reported for algae, invertebrates, fish and plants, respectively, which indicates no ecological risk. However, the potential for synergistic or additive toxicity to aquatic organisms and/or other toxicity effects, which has not yet been studied, cannot be ruled out. According to the inventory of chemicals defined as endocrine disrupters compiled by the Institute for Environment and Health (IEH, 2005), which includes a total of 966 compounds, carbamazepine (detected in all samples at concentrations up to 179 ngL^{-1}) affects circulating thyroid hormones and sulfamethoxazole (detected in all samples at concentrations up to 119 ngL^{-1}) alters thyroid function.

4.4.2. Levels of oestrogens

Among the group of target oestrogens, oestrone and oestrone-3-sulphate were the only analytes found at the Llobregat basin surface waters and at very low concentrations (in the low ngL^{-1} range). Table 4.3 lists the LODs achieved for the various compounds monitored. Oestrone concentrations, measured in all but one sample, ranged from 0.82 to 5.81 ngL^{-1}. The highest levels were detected at site A (2.38 and 5.81 ngL^{-1} in the first and second sampling campaign, respectively) and site B (2.80 ngL^{-1} in the first sampling campaign). These levels are within the range of those (1 to 10 ngL^{-1}) from which oestrogenic effects can be expected (1 to 10 ngL^{-1} depending on the oestrogenic assay used) (Petrovic et al., 2004). Conversely, the most potent oestrogenic compounds (estradiol, ethynyl estradiol, and diethylstilbestrol) were not found in any of the samples analysed.

Table 4.3. Method detection and quantification limits of the various target oestrogens in the on-line SPE-LC-QqQ-MS/MS method

Compound	LOD (ng L-1) SRM 1	LOQ (ng L-1) SRM 1	LOD (ng L-1) SRM 2
Oestriol-3-sulphate	0.05	0.12	0.23
Oestriol-16-glucuronide	0.16	0.41	0.22
Estradiol-17-glucuronide	0.23	0.62	0.67
Oestrone-3-glucuronide	0.14	0.38	0.35
Oestrone-3-sulphate	0.02	0.06	0.10
Oestriol	0.49	1.32	0.62
Estradiol	0.53	1.42	0.65
Ethynyl estradiol	2.20	5.86	2.90
Oestrone	0.12	0.32	0.35
Diethylstilbestrol (DES) [a]	0.30	0.79	0.94

[a] For DES, SRM1 refers to the first peak and SRM2 to the second peak of the first transition

Oestrone-3-sulphate was found in 80% of the samples and reached values between 0.25 and 1.46 ngL^{-1}. Additionally, oestriol-3-sulphate was detected at very low levels in some samples but its presence could not be confirmed because the concentration measured using the most abundant SRM transition was lower than the method limit of detection achieved with the second transition. UPLC-TOF-MS could not be used in this case for confirmation due to insufficient sensitivity. Oestrogenic activity for conjugated oestrogens is lower than for the free oestrogens; levels found seem to have no risk for the environment.

4.5. Conclusions

The combination of two LC-MS techniques was used to unequivocally detect and quantify levels of 38 compounds in surface waters of the Llobregat River basin. LC-QqQ-MS/MS was used for detection and quantification because of its high sensitivity and possibility of confirmation when two transitions of the parent ion to product ions are recorded. When a second transition could not be selected, the accurate measurement of the mass of the base ion was performed using UPLC-TOF-MS for confirmation. This approach was used to confirm the presence of ibuprofen and gemfibrozil in all samples and of diclofenac, atenolol, sotalol and metoprolol in samples showing high levels of these compounds. Confirmation of pravastatin and ofloxacin by UPLC-TOF-MS was not possible due to insufficient sensitivity. The main disadvantage of this solution is the extra cost related to the performance of two analyses. This problem could be partially solved by solely analysing the samples containing those analytes for which extra confirmation is needed.

Results from the monitoring performed confirmed the presence of drugs of high consumption as expected in a densely populated Mediterranean basin. Significant levels, higher in general than those previously reported in the literature, were found for the ß-blockers metoprolol and sotalol, the antibiotic ofloxacin and the lipid regulator gemfibrozil. Within the group of oestrogens, only oestrone and its conjugated derivative oestrone-3-sulphate were confirmed to be present. Oestrone levels were in some sites close to those considered sufficient to cause oestrogenic effects in aquatic organisms.

Two sites out of the eight monitored showed distinctly high concentrations of both classes of compounds; however, their waters are diverted to reach the river at locations close to the mouth and downstream of the inlet of the Sant Joan Despí DWTP, which supplies water to a large part of the Barcelona metropolitan area, in order to protect the quality of the source water. Along the river, the contamination load was observed to increase from upstream to downstream.

This study confirms the presence of some of the target compounds in concentrations that could lead to a potential risk to the environment and human health. Further studies on the risk of these compounds need to be undertaken.

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5

Evaluation of an automated luminescent bacteria assay for in-situ aquatic toxicity determination

5. Validation of a Water Quality Monitoring Platform at Barcelona Drinking Water Treatment Plant

5.1. Abstract

The use of early warning technologies that can provide real-time information on water quality will increase in the following years for water protection against contamination. The main objective of the work is to propose and validate a methodology for the on-line monitoring of surface water quality at the intake of a Drinking Water Treatment Plant (DWTP) adapted to operators' needs.

For that purpose, a biological toxicity monitor using luminescent bacteria (TOXcontrol^{TM}) coupled to a UV-VIS spectrophotometer probe is being tested online at the intake of the Barcelona DWTP. The plant is located downstream in the Llobregat River, receiving discharges from more than 30 WWTPs and occasional accidental spills from industries, so a real-time control is crucial for safety reasons.

On-line measurement of several paremetres (TSS, COD, TOC, NO3-) in one single UV-VIS probe could help to establish relationships between these paremetres and characterise surface water before entering the plant. Toxicity is being measured by decreases of the luminescence of *Vibrio fisheri* bacteria and an alert signal is established. A SPE concentrator prototype is also being tested to increase sensitivity of this biomonitor to toxic substances. This combination will be validated for pollutants more commonly found in the tested area and those posing major risk to water production (pesticides, personal care products, surfactants etc.)

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5.2. Introduction

Water bodies' quality monitoring is essential nowadays, but not only for the preservation of the ecological status. Final uses of water, especially for irrigation, industrial application or potable purposes, demand good quality. Treatment technologies are implemented when quality does not fulfil the requirements for these specific uses. The performance of these technologies and quality of produced water are also very dependent on the characteristics of the resource at the intake.

Actions have also been implemented to improve the quality of these natural bodies. The impact of these preventive and/or corrective actions should be monitored to follow-up their efficacy and to fulfil regulatory standards.

At a European level, quality requirements to be met are mainly addressed by the Water Framework Directive (WFD, 2000/60/EC)[1] and the Priority Substances Directive (2008/105/EC)[2] where Environmental Quality Standards (EQS), that is, concentration, and Annual Average (AA), that is, total loads, are established for a list of 33 pollutants. Performing laboratory analysis of these 33 pollutants represents a large number of analyses and it should be taken into account that it is not enough to measure whether or not the priority substances concentration is below the EQS or not because this is not necessarily representative of the water status. Moreover, spot sampling campaigns, the most common approach for analysing these compounds, are costly and labour-intensive and not sufficient to obtain an accurate picture of the chemical and biological status of water quality.

For all of these reasons, new tools need to be established to obtain all the information needed.

One of the most sensitive uses of natural waters is their treatment for drinking purposes. Source water needs to comply with the requirements that make it suitable for treatment in the drinking water plant facilities. European and national legislation has also been established to protect the health of drinking water consumers. Drinking Water Directive (98/83/EC)[3] and recent application of Water Safety Plans (WHO Guidelines[4] or ISO 22000[5]) imposes strict regulation on the monitoring of water quality indicators, mainly aimed at protecting human health. Regulatory and technology requirements make it necessary to monitor water at the intake of treatment plants.

5.3. Location. Case Study

The Barcelona area (NE of Spain) is supplied with drinking water from different sources. Water scarcity situations, typical in the Mediterranean area, require complex management taking into account the availability of different water sources: The Llobregat and Ter Rivers, groundwater, sea and brackish water, all combined, in different blends, according to the season of the year and the exact location in the Barcelona area.

Although having this complex system of water supply, the main source is surface water coming from the Llobregat River. Previous studies and monitoring campaigns show the presence of a significant number of families of pollutants[6-8]. Some physico-chemical paremetres are continuously monitored. The values of these paremetres exhibit significant variations, especially due to the seasonal effects. Figure 5.1 shows information on the flow variation at the intake of Sant Joan Despí Drinking Water Treatment Plant (DWTP). The uneven distribution of rainfalls during the year causes sudden alterations of river characteristics, especially flow and turbidity due to runoff in heavy rain events.

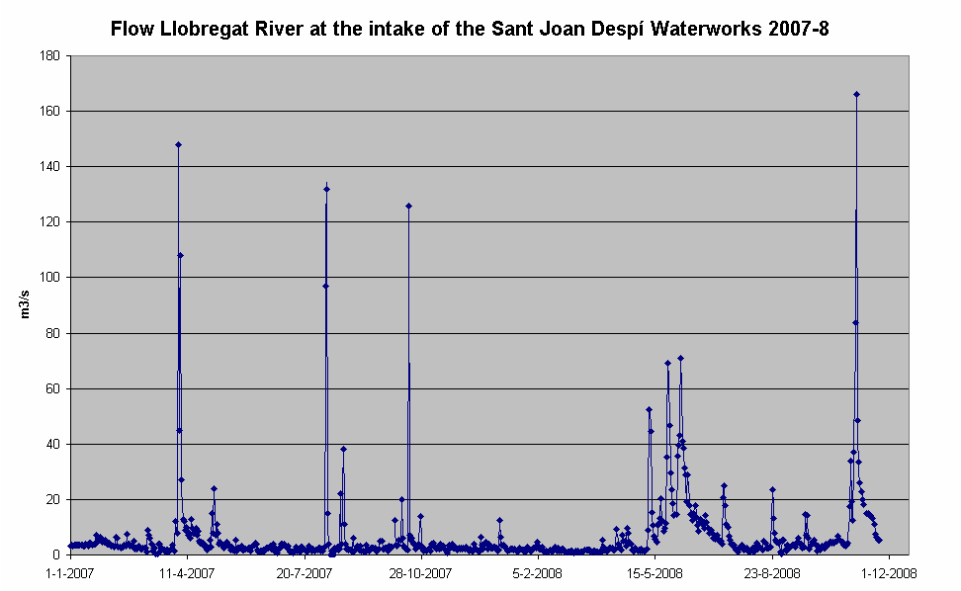


Figure 5.1.Seasonal flow variation of the Llobregat River at the Sant Joan Despí DWTP

A sudden alteration of quality paremetres could be interpreted as an episode of accidental spill or illegal discharge into the river, not only impacting on ecosystems but creating a situation where polluted water could enter the water treatment plant, potentially affecting the quality of drinking water. If this situation occurs, intake from the relevant surface water is closed and groundwater is pumped from the aquifers in the area.

5.4. VIECO project

In order to successfully achieve this objective, a monitoring platform had to be designed. The platform integrates off-line and on-line measures and in-line integrating sampling tools in order to achieve the detection of a wide range of different paremetres and to have integrated responses according to water quality.

Initially, the project was focused on the integration of these new sensing technologies (including chemical and bio-sensors) with the existing ones, in order to obtain automatic and autonomous detection of different paremetres and to have integrated responses according to water quality. In a second step the monitored data are now being integrated to establish a relationship between field ecological observations and measured paremetres related to chemistry, toxicity, etc. and additionally to distinguish the contribution of unknown (not measured) causes. The platform will provide a low-cost management tool for providing different levels of environmental information in surface waters, with a high degree of integration of data processing and interpretation of whole results.

5.4.1. Objectives

The main objective of this project is to contribute to the exhaustive characterisation and further enhancement of water quality by developing and validating a cost-effective monitoring platform to provide environmental indicators of ecological, chemical and biological status of surface waters. Validation will be performed at the main DWTP in Barcelona.

This objective will be achieved by providing the platform with emerging tools and validating results using traditional methods. Environmental indicators will be offered to operators and the government relating to the water ecological status, integrating data provided by the tools that have been validated, thereby helping to implement the WFD.

5.4.2. Actions and resources involved

In order to reach the above mentioned objective, the following sets of actions are being carried out:

5.4.2.1. Definition of the off-line chemical sensing platform

Analytical methods have been optimised for the analysis of a selection of priority and "emerging" compounds. Liquid chromatography coupled to tandem mass spectrometry was the technique selected. Different methods for detection and quantification were developed for pesticides, pharmaceuticals and fullerenes. A list of contaminants was selected on the basis of: a) their high use and/or production, b) their significant aquatic toxicity, c) their fate in the aquatic environment (high stability and low biodegradability), d) their poor removal during activated sludge wastewater treatment.

5.4.2.2. Integration of sensors for the on-line bio and chemical sensing platform

In this task, on-line chemical and biological sensors are currently being validated for their integration into the monitoring network stations.

To do this, a biological toxicity monitor using luminescent bacteria (TOXcontrol™, microLAN, Netherlands) coupled to a diode array UV-VIS spectrophotometer probe is being tested (spectro::lyser™, s::can, Austria). The on-line measurement of several paremetres (TSS, COD, TOC, NO3-) using a single UV-VIS probe could help to establish relationships between these paremetres and characterise surface water before entering the plant. Toxicity is being measured by the decrease of the luminescence of *Vibrio fisheri* bacteria and an alert signal being generated. A Solid Phase Extraction (SPE) concentrator prototype is also being tested to increase the sensitivity of this biomonitor to toxic substances. This combination is being assessed for pollutants more commonly found in the studied area and those posing a major risk to water production including pesticides, personal care products, surfactants, etc.

A combination of complementary techniques will enable the verification of alarm signals, thereby reducing false alarm rates. Where a chemical analytical monitoring system identifies and quantifies specific water contaminants, biomonitoring gives an indication of the total quality, including the effects of unknown toxic substances.

Equipment for indirect on-line measuring of the Biological Oxygen Demand (DBO Optosen 50™, Interlab, Spain) based on the measurement of oxygen consumed by bacteria immobilised on a plate, will be validated not only at the intake of the DWTP but at the effluent outlet of a Wastewater Treatment Plant (WWTP).

5.4.2.3. Integration of in-line sensing tools

Methodologies based on passive samplers have been developed for the in-line integrated monitoring of water quality as they provide more realistic results than conventional methods. Passive samplers are introduced into surface water streams and they adsorb certain chemicals during a period of time (normally between 2 and 4 weeks), commonly through a diffusion-limiting membrane. Afterwards the samplers are removed and taken to the laboratory, where target compounds are extracted and quantified. As a result, these devices calculate a time weighted average concentration (TWA), which is more representative than results of conventional methods using spot samples, because they mimic how these pollutants are taken by animals and plants and how they bioaccumulate. In addition to this, as they integrate concentration over a period of time, they provide an average, not a snap shot of the situation when a sample is taken.

The diffusive gradient in thin film (DGT) devices was tested for the analysis of metals. Polar Organic Chemical Integrative Samplers (POCIS) were tested for the analysis of polar compounds such as pharmaceuticals, pesticides and surfactants, while an automatic device for constant flow integrative analysis (CFIS) was developed and tested for non-polar organic compounds like PAHs, PCBs, pesticides, phthalates and surfactants.

5.4.2.4. Implementation of the biomonitoring platform for the assessment of pollution induced effects on biofilm communities

A bio-monitoring platform was developed using field diagnostic tools for the estimation of water quality. The aim of this task was focused on the implementation of tools for a community-based toxicity assessment of site-specific toxicants by integrating structural, physiological and functional paremetres of microbenthic communities and community-based ecotoxicological assessment approaches. Changes in community structure (biomass, taxonomy and metabolic profiling) were studied under different toxicity exposures. The effects on functional paremetres from biofilms will be assessed through a variety of methods.

5.4.2.5. Risk evaluation of toxicity in aquatic ecosystems

The proposed task will be focused on the integration, at river basin scale, of several paremetres measured by sensors, classical analytical chemical methods and bioassays to define indicators to assess the impact and the effects of ecological field observations. Thanks to this methodology, it will be possible to relate the field ecological observations (e.g. abundance of species) to paremetres related to habitat, chemistry,

toxicity, effluents and to identify the contribution of unknown (not measured) causes. The correlation between the monitored and quantified paremetres within the project versus the observed ecological paremetres will be carried out using generalised linear models (GLM) and a flexible quadratic model will finally identify the influence of each group of variables on the final observed value and, thereby, attribute the effects to paremetres.

5.4.3. Results on validation of on-line sensors

5.4.3.1. Spectrophotometer probe

We tested the diode array UV-VIS spectrophotometer probe in the first stage of the project, in the surface water entering the DWTP. Measures were scheduled automatically every 10 minutes. The probe was connected to an air compressor to clean the window every 5 measurements. The profile of the spectrum from 200 to 750 nm was recorded during each measurement. The calibration model showed values from pre-established paremetres with the information obtained from the spectral profile. In this case, on-line measurements of several paremetres: - Turbidity, Total Organic Carbon (TOC), nitrates and SAC-254 were performed. Correlations were established between values obtained from the probe and the analysis performed in the laboratory. This comparison will give us information on the performance of the instrument and it will be used by the probe itself for local calibration.

The main advantages of using a multiparametric probe are: compilation of information about several paremetres using a single technology resulting in a lower overall cost for purchase and maintenance; real-time continuous monitoring of the water at the intake (potentially applied to other steps in the process of drinking water treatment), making it suitable to be used as an alert system; recording of the whole spectrum from 200 to 750 nm to enable us to add new paremetres or compounds and extract concentration values from the fingerprint.

The main difficulty found when validating the probe was the unstable conditions of the Llobregat River. The water matrix changes quickly in Mediterranean rivers (e.g. turbidity can change from 30 NTU to 1000 NTU in a few hours). The probe was calibrated for a concentration range of paremetres that were narrower than the range found in real conditions. This meant that in certain episodes (heavy rains), data was out of range and could not be recorded. The year the validation process took place (2010) had extraordinarily high rainfall, so extreme results, especially turbidity values, occurred quite frequently. Figure 5.2 shows turbidity values from November 2009 to June 2010 (values above 300 NTU are not shown).

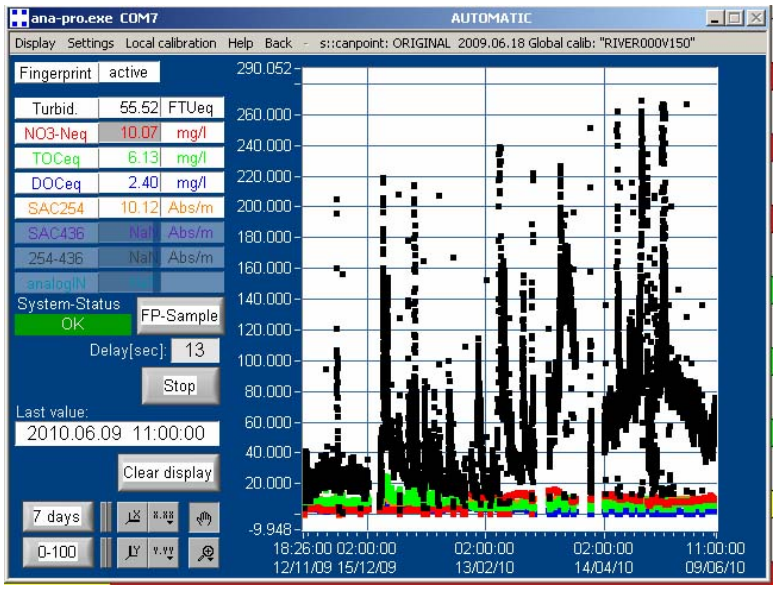


Figure 5.2. Screen shot of spectrophotometer probe software showing variations in turbidity values

5.4.3.2. Biological toxicity monitoring

A biological toxicity monitor using luminescent bacteria is being tested. The equipment is completely automated. Measurements of the incoming flow of water are carried out every 30 minutes with a quasi-real detection of the global toxicity of the compounds present in the water. Toxicity is being measured by the decrease of the luminescence of *Vibrio fisheri* bacteria when being in contact with the sample. The exposure time has been set at 15 minutes. An alert signal can be established at a certain value of light inhibition.

At the Llobregat River, it was found that the background levels of toxicity are typically in a range of ± 20% of light inhibition. As we stated earlier, the water matrix changes due to weather conditions. When turbidity is high, we can see some positive toxicity due to the decrease of light arriving at the photomultiplier light detection system in the equipment (Figure 5.3). In low turbidity episodes, toxicity values are negative because of the high content of nutrients in Llobregat water that results in an increase of the metabolism of the luminescent bacteria and therefore, in the emission of light.

In one of the experiments performed, the toxicity monitor was used to analyse, in off-line mode, 6 samples of surface water at the intake of DWTP plus another 6 samples of treated wastewater at Abrera WWTP, discharging to the Llobregat River upstream of the DWTP intake. A system was designed using a pump so the same sample flowed in a loop and it could be measured several times. Having different

measures of the same water gave us information on the repeatability of the method. Different aliquots from the same sample were analysed by a new prototype version of the toxicity monitor to compare the performance of both instruments; and also by certified methods using *Vibrio fisheri* (Microtox™) and *Daphnia magna* (planktonic crustaceans).

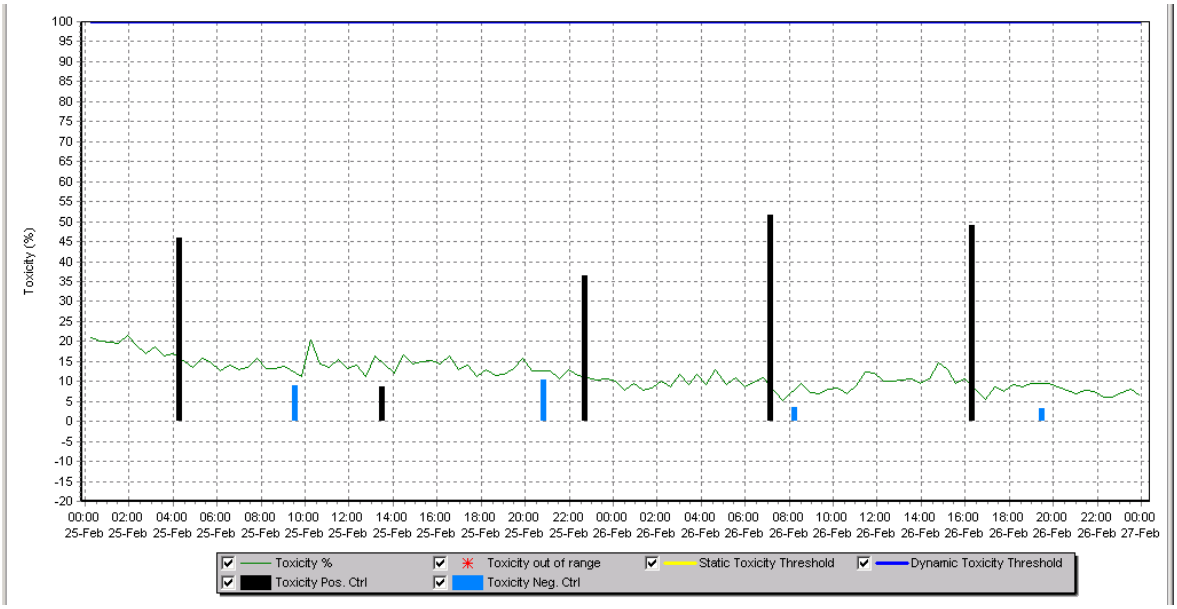
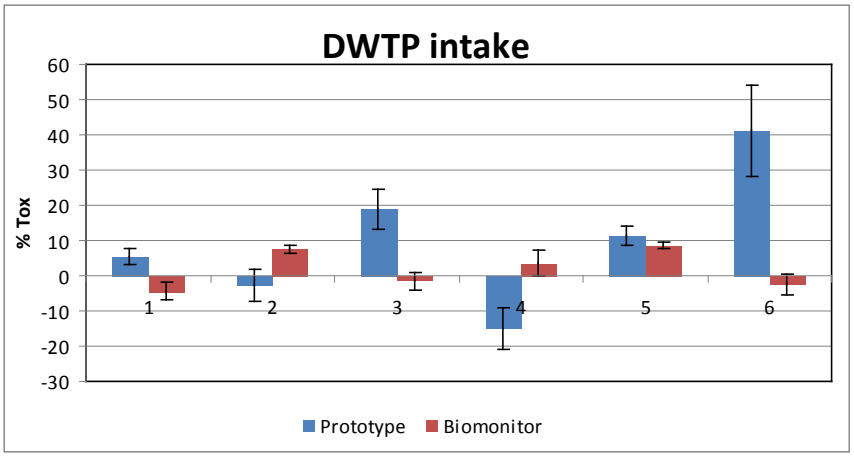


Figure 5.3. Toxicity background levels relating to episodes of high turbidity

Figure 5.4 shows that, although some differences can be seen by comparing the two versions of equipment, no significant inhibition was shown, so no toxicity of any of the samples could be reported. Checking the dispersion of data, repeatability was better in the biomonitor during validation, as it is a more mature version than the new prototype. Analysis performed with Microtox™ and *Dahpnia magna* also showed no toxicity.

The equipment shows the advantage of detecting global toxicity of some of the priority pollutants (metals and pesticides) that causes inhibition in *Vibrio fisheri* bacteria. It can operate as an alert system for intake protection and the maintenance work is low (once a week). The main disadvantage of the equipment is that bacteria are not sensitive enough to detect the presence of toxic compounds at the levels needed to comply with legislation. To help to solve this problem, a Solid Phase Extraction (SPE) concentrator prototype is also being tested to increase sensitivity of this biomonitor to toxic substances.

a)



b)

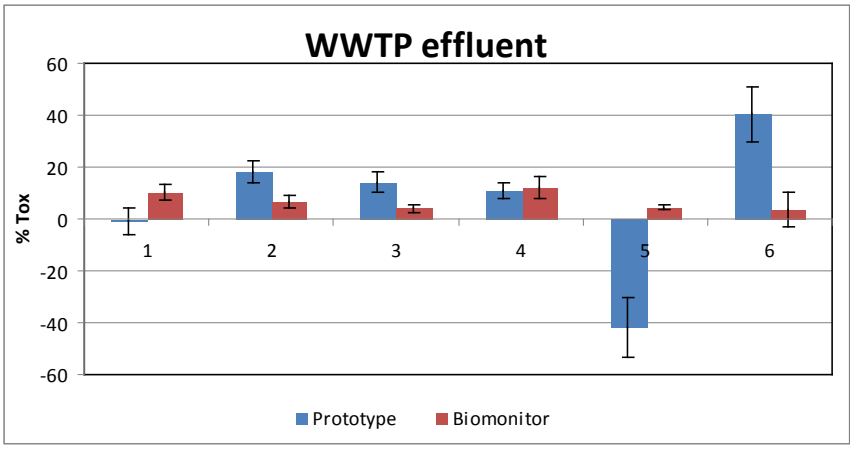


Figure 5.4.Comparison between two pieces of equipment (prototype and biomonitor) for surface waters (a) and treated wastewaters (b). Samples are numbered from 1 to 6 relating to consecutive weeks

5.4.4. Future work

Once the pieces of equipment have been validated for real sample analysis, experiments will be performed for a selection of ten target compounds. These substances have been selected according to their occurrence in Llobregat River and the potential to show toxicity. The following ten compounds will be used for testing: Terbutylazine, Diazinon, Dimethoate, Diuron, Propanil, MCPA, Nonylphenol, LAS, Triclosan and Diclofenac.

5.4.4.1. Recovery tests for SPE concentrator

In order to test the performance of the SPE module for increasing concentrations of our target compounds, so they can show toxicity when analysed by the biomonitor, the following experiment has

been designed. HPLC grade water and surface water (filtered 0.45µm) will be spiked with a standard mixture of the ten target compounds. Samples will be spiked at 0.2 mg L^{-1} and 0.2 ng L^{-1} so after concentration (from 500 mL to a final volume of 10 mL), final concentrations are expected to be 10 mg L^{-1} and 10 ng L^{-1}. Two different cartridges will be tested: original ones and self-prepared (using OASIS HLB Waters™ material). Moreover, three replicates plus blank will be prepared for each situation.

5.4.4.2. Dose response curves

The concentration that causes 50% of inhibition (EC50) will be calculated according to the "dilution series procedure" (TOXcontrol™) for each target compound. In this procedure, different concentrations of the same compound are tested automatically to obtain the curve concentration-response that will show information for the EC50 calculation. Due to the low solubility of some of the target compounds, solutions will be prepared in DMSO 0.2%. Such a low concentration of DMSO has been confirmed to show no response to *Vibrio fisheri*.

5.4.4.3. Toxicity tests for the target compounds

Taking into account information on recovery and toxicity obtained from previous tests, experiments will be designed for testing the response of the toxicity monitor when dealing with different mixtures of our target compounds after pre-concentration. Synergistic or antagonists effects could also be checked. The influence of matrix on the response will be also an important issue to assess.

5.5. Conclusions

Water resources quality monitoring is a mandatory issue for ecological and health reasons. New tools should be integrated into water quality monitoring programmes. The VIECO project develops methodology and validates technology before their routine use. The special characteristics of a case study (Llobregat River) pose a challenge to the implementation of some technologies, especially those operating on-line.

Weather conditions can cause alterations in paremetres such as turbidity, making it difficult to measure this or other paremetres when previous calibration is needed or when optical measures are implied (absorbance or luminescence).

Sensitivity is another important obstacle to overcome when on-line instruments are used. Pre-treatment of the sample can augment concentration of pollutants, but could increase the response time or imply the need for the implementation of some manual processes.

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6

Validation of a Water Quality Monitoring Platform at Barcelona Drinking Water Treatment Plant

6. Evaluation of an automated luminescent bacteria assay for in-situ aquatic toxicity determination

6.1. Abstract

A new system for monitoring toxicity TOXcontrol™ (MicroLAN BV, The Netherlands) was used to assess the toxicity of a selection of priority or emergent compounds in the laboratory. In this study, inhibition curves and EC50 - Effective Concentration causing 50% inhibition- of selected compounds (including pesticides, pharmaceuticals, surfactants and metals commonly detected in surface or drinking waters) were determined. This new technology is based on the measurement of *Vibrio fischeri* bioluminescence inhibition (ISO 11348). The main advantage of this equipment, compared to other laboratory assays, is the full automation of the procedure. The instrument can be operated online in a simple, rapid and reproducible way. We studied the variability of the results obtained with the TOXcontrol™ biomonitoring system. A comparison with standardised technology based on *V. fischeri* (Microtox™) and additional test with *Daphnia magna* for selected organic compounds is presented. The results show that the methodology based on the TOXcontrol™ system being validated is accurate and reproducible enough, thereby enabling this system to be used as an on-line automatic alert system to detect abnormal concentrations of toxic compounds.

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6.2. Introduction

Legislation related to the preservation of quality of water bodies is becoming more stringent both at national and international level. In the European context, the Water Framework Directive (WFD) (2000/60/EC) is an example of the new risk-based attitude adopted in terms of environmental impacts. The WFD requires both a good chemical and biological status of water bodies; therefore new monitoring assays to detect changes in water quality at short notice are required. Although a list of priority substances to be determined in surface water bodies was established by the Daughter Directive (2008/105/EC) according to their harmful potential, other substances exist that are not regulated at the moment, called emerging contaminants, which are liable to affect living organisms, although their concentrations are not routinely measured. A large number of studies have been performed with the aim of identifying those contaminants present in water flows (Von der Ohe et al., 2011).

The objective of the Drinking Water Directive (DWD) (98/83/EC)is to protect human health in the European Union and to make sure that water is healthy and clean. For this purpose, DWD sets standards for the most common substances that can be found in drinking water. In the DWD a total of 48 microbiological and chemical paremetres must be monitored and tested regularly. The thresholds of these substances (including pesticides, metals, bacteria, etc.) are based on the potential detrimental effects to organisms.

The chemical status of water bodies is determined in most cases by spot sampling campaigns and laboratory determinations. This off-line methodology is slow and in some cases ineffective to respond to sudden quality changes as a result of possible contamination. There is a need to use some alternative monitoring tools to complement traditional ones in order to provide a comprehensive overview of water quality. Biomonitoring protocols use sentinel species, defined as any living organism used as an indicator of the presence of a pollutant or the toxicity of a contaminant (Amiard and Amiard-Triquet, 2008). Biological analysis with the help of different biosensors is considered a highly informative testing system, since the knowledge about the chemical characteristics of pollutants does not always provide sufficient information about their toxicity and danger for living organisms (Tsybulskii and Sazykina, 2010).

Toxicity tests are one example of a commonly used biomonitoring tool. In this case, the biological response of a test organism is measured as the result of the combined effect, including antagonism and synergism, of the mixture of all potential contaminants contained in water. One of the most common biosensors used for the risk assessment in aquatic environment is based on the inhibition of luminescence produced by marine bacteria *Vibrio fischeri*. The use of this bioluminescence based assay has been standardised (ISO 11348-3) for regulatory purposes because of its sensitivity and short time required to

perform the test (Coz et al., 2007). Toxicity is usually represented as EC50, i.e. effective concentration of the tested chemical at which 50% of luminescence inhibition is observed.

Since the early 80s, many studies have been performed to determine the toxicity of different families of chemicals in the laboratory. In one of these studies, the drugs investigated (ibuprofen, ketoprofen, naproxen, diclofenac, salicylic acid and gemfibrozil) showed very similar EC50 values when comparing two techniques using the luminescent bacteria assay (14 - 36 mgL^{-1} for Microtox™ and 12 - 43 mgL^{-1} for ToxAlert™) (M Farré et al., 2001). Other comparisons performed with surfactants showed greater variability (0.36 - 127 mgL^{-1} for ToxAlert™ and 0.40 - 379 mgL^{-1} for Microtox™) (M. Farré et al., 2001). Antibiotics showed a moderate toxicity on *V. fischeri* and no significant effects at the maximum concentration tested relating to water solubility were observed, but the compounds atrazine, simazine, glyphosate, deltametryn and leucomalachite green showed EC50 values greater than 10 mgL^{-1}, and therefore they were classified as harmful, according to the Global Harmonised System of classification (UNECE, 2011; Hernando et al., 2007). EC50 values have also been determined for triclosan(0.28 mgL^{-1}) and methyl triclosan (0.21 mgL^{-1}) (Farré et al., 2008).

In other studies, EC50 values for individual metal added in the ionic form were obtained for cadmium, chromium, copper, lead and zinc using *V. fisheri* at an exposure time of 30 min. Values ranged from 0.12 to 13.8 mgL^{-1}(Guéguen et al., 2004). In another study, the toxicity of the 13 priority pollutant metals and non-metals was evaluated using the Microtox™ chronic toxicity test. Among the metals, beryllium was found to be the most toxic in the test while thallium was the least toxic (Hsieh et al., 2004). The toxicity of arsenic, cadmium, lead, and mercury has been tested individually and as a composite mixture using the Microtox™ bioassay. Among the individual metals and non-metals tested, in the ranking of toxicity mercury was in first place, followed by lead, cadmium and arsenic (Ishaque et al., 2006). More tests have been performed in assessing toxicity of metals based on this bacterium (Codina et al., 1993; Cho et al., 2004; Rosen et al., 2008; Tsybulskii and Sazykina, 2010). The response of luminescent bacteria to mercury compounds has also been investigated (JM Ribo et al., 1989).

Further studies have been performed to assess the performance of different sensors. Ten toxicity sensors utilising enzymes, bacteria, or vertebrate cells were compared to rapidly identify toxicity in water samples containing one of 12 industrial chemicals. Microtox™ was the highest scoring in the ranking, responding to 6 out of 12 compounds (van der Schalie et al., 2006). Another study performed a comparison between *V. fischeri, Selenastrum capricornotum and Daphnia magna* tests. A selection of pesticides, antifouling agents and pharmaceuticals were tested. *D. magna* was the most sensitive test. *D. magna* and *V. fischeri* both showed discriminatory ability to separate compounds in different toxicity categories (Hernando et al., 2005). In a different study, the inhibitory effects of 81 chemicals, after 5 min contact time, were calculated at eight concentrations using three commercial assay systems based on the luminescent

bacteria toxicity assay (ToxAlert™, Microtox™ and LUMIStox™). Only five compounds gave EC50s that varied more than three-fold between assays (Jennings et al., 2001). Bioluminescent bacteria have been applied frequently to monitor toxicity in several environmental applications such as wastewater, seawater, surface and ground water, tap water, soil and sediments, and air (Girotti et al., 2008; M.C. Riva et al., 2007). However these tests were based on discontinuous samples and provide only a partial response in terms of compliance with WFD and related legislations.

The objective of the study was the testing of the automated equipment TOXcontrol™ for measuring toxicity of contaminants that can be found at surface and drinking waters. We assessed the response of the equipment in the laboratory compared to a selection of contaminants. The response for the organic compounds was compared against the response of toxicity tests performed with Microtox™. As it is a non-specific technique for measuring global toxicity, it was important to perform tests in the laboratory with water spiked with single analytes so a specific response to one compound could be obtained. Only one reference was found concerning the validation of on-line toxicology sensors, in which TOXcontrol™, was tested in combination with a spectrophotometer for the monitoring of sodium cyanide, azinphos-methyl, sodium fluoroacetate and difenacoum in surface waters (Appels et al., 2007). Additionally, the sensitivity of *V. fischeri* for selected organic compounds was compared against the sensitivity of *D. magna*.

Firstly, contaminants were selected taking into account their occurrence in semi-arid basins, such as the Llobregat River (SE Spain) where a high contribution of treated wastewaters discharges in the total flow of the river is expected, as the low flow makes dilution factor almost negligible. The pollutants can become a potential risk to the receiving bodies and, in addition, to the production of drinking water (Gasperi et al., 2008; Muñoz et al., 2009). Secondly, information on the possible presence of metals in drinking water according to their potential for being transferred from the corrosion of internally unprotected metallic water pipelines was taken into account for the metals selection (Imran et al., 2009).

We performed experiments to assess the aquatic toxicity of a selection of target compounds. These substances were selected in view of their occurrence in surface water, especially in semi-arid regions where the water stress leads to low flow rates and a higher concentration of dissolved pollutants, and their potential to show toxicity (González et al., 2012) and their possible presence in drinking water mainly due to migration of pipe material (Veschetti et al., 2010).

6.3. Materials and methods

6.3.1. Reagents and standard solution preparation

Chemical standards for terbuthylazine, triclosan, dimethoate, sodium dodecylbenzene sulphonate (SDBS), diazinon, sodium diclofenac, nonylphenol, propanil, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and iron (III) sulphate hydrate were purchased from Sigma Aldrich (St. Louis, MO, USA). Standards for chromium (III) nitrate nonahydrate, copper (II) sulphate pentahydrate, lead (II) nitrate and nickel (II) sulphate hexahydrate were purchased from Merck (Darmstadt, Germany). HPLC water and dimethyl sulfoxide (DMSO) were also purchased from Sigma-Aldrich. The freeze dry luminescent bacteria *V. fischeri*, cultivation media, zinc sulphate (2500mg/l) and 20% sodium chloride were supplied by MicroLAN (Waalwijk, the Netherlands). Stock solutions were obtained by dissolving metal salts in HPLC water and organic compounds in DMSO (0.2% v/v). The pH of solutions was not adjusted but it was monitored and no sudden changes of pH were reported.

Luminescent marine bacteria of the species *Vibrio fischeri* (NRRL B-11177) for the Microtox® determinations were obtained from SDI (Strategic Diagnostics Inc. Newark, DE USA.) Luminescent bacteria for ToxControl® determinations were obtained from Microlan B.V. Waaljik. NL. *Daphnia magna* used as test organisms were obtained from a CRIT-UPC maintained culture.

6.3.2. On-line Toxicity monitoring

The instrument for online monitoring of toxicity of water samples was the ToxControl™ Toxicity Monitoring System manufactured by Microlan, B.V.

TOXcontrol™ is an advanced automatic on-line water toxicity monitor based on the use of luminescent bacteria (*V. fischeri)* to give an indication of the toxicity of the contaminants in water as a function of the emitted light. After the mixing of the luminescent bacteria and the water sample, the toxic material in the sample would alter the metabolism of the bacteria. The decrease of light intensity is directly proportional to the concentration of toxic substances in the sample.

The equipment works on the same basis as the certified methodology for the analysis of toxicity with *V. fischeri* (ISO 11348-3) but adapted to automatic equipment. The analyser inside the equipment is designed to work in two parallel lines. A diagram of the modular parts can be seen in Figure 6.1. While one of the lines prepares the mixture of the bacteria solution with sample water and measures the effect of that sample on bacteria, a second line uses reference water instead of sample water, as the output data is a

relative measurement of the light emitted by the first line compared to the second one. Before performing the measure, dry frozen bacteria need to be re-hydrated by adding cultivation media and mixing under controlled temperature conditions for several days (2 days at 2ºC, 20 hours at 20ºC, 3-5 days at 4ºC for maintenance until used).

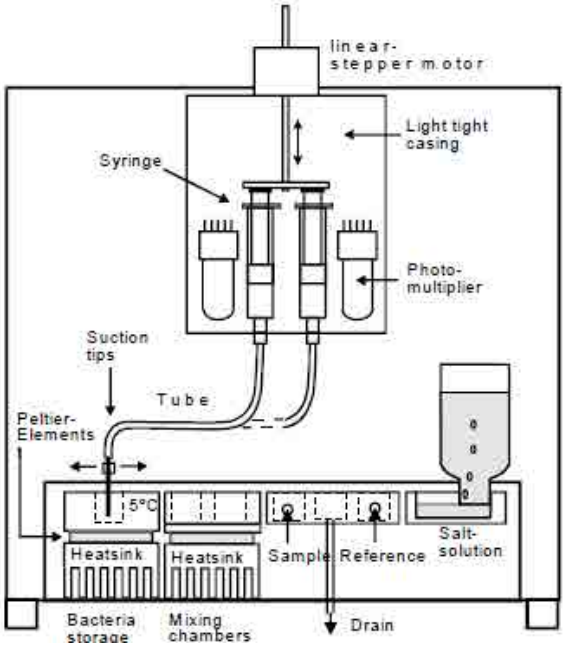

Figure 6.1. Diagram to represent the functioning of the automated TOXcontrol ® toxicity monitoring system

The bacteria module, which always maintains the inside temperature at 5ºC, is filled with luminescent bacteria suspension. The luminescent bacteria cannot suffer constant alterations in temperature. After the luminescent bacteria are taken out of the cup, they are mixed with a sample and a sodium chloride solution (2g in 100ml of water) in the mixing module. The temperature is kept constant at 15ºC during incubation time (15 min or 30 min). EC50 values were calculated according to "dilution series procedure" of the TOXcontrol^{TM} instrument for each target compound. In this procedure, different concentrations of the same compound were tested automatically to obtain the curve concentration-response that will show information for the EC50 calculation.

According to this procedure, 50 µl of bacteria suspension are mixed with 5 ml of the sodium chloride solution for the preparation of the bacteria solution. This first part of the process takes 5 min. Measurements of luminescence are done for assuring activation of bacteria. Then a volume of standard working solution, instead of the real sample, is taken from a vial and diluted in another volume of the sodium chloride solution to constitute a final volume of 5 ml of diluted sample. When both solutions are mixed, a final volume of 10 ml of bacteria in contact with the analyte is incubated for 15 or 30 min.

Variables to be specified in this procedure are: the volume of sample to be taken, the number of dilutions per series (being 0 ml of sample the first point, the chosen volume of sample as the second point, and doubling the volume of sample for every successive point), and the number of series (repetitions) to be performed.

Due to the low solubility of some of the target compounds, solutions for organic analytes were prepared in DMSO 0.2% v/v. Such a low concentration of DMSO has been shown during the preparation of the experiments to show no response for *V. fischeri*(Hernando et al., 2007). A series of five concentrations of each test solution were prepared and the measures were performed in triplicate.

A quality control of the performance of the tests was executed. Positive and negative controls of the measurements were done before and after each series of measurement. The negative control was done using reference water. Measurement was accepted if the toxicity value was between -3 and 3%. A positive control was done using zinc sulphate (2500 mgL^{-1}). If toxicity was over 60%, the series of measurement were accepted. If the negative or positive control was out of range, the series of measurements were excluded and the test repeated.

The sigmoidal inhibition curves were calculated with the help of the Prism 4 software (GraphPad Software Inc.).

6.3.3. The Microtox™ Toxicity assay

The experimental procedure to determine toxicity using the Microtox™ Toxicity Analyser has already been described (Kaiser and JM Ribo, 1988) and it is based on the standardised method for the analysis of toxicity using *V. fischeri* (ISO 11348-3). The toxic effect of an aqueous sample is determined as the concentration of the sample causing a 50% reduction on the light emitted by the bacteria, after a pre-determined exposure time (5 min, 15 min or 30 min). For regulatory purposes an exposure time of 15 minutes was established.

In this procedure the freeze-dried bacteria is reconstituted with water, to provide a stock suspension of test organisms that is kept at 5ºC and used to perform the test. The luminescence of the bacteria after exposure to the toxic sample is compared with that of a control at the test temperature (15ºC). A correction factor has to be applied due to the loss of luminescence of the control (reduction in light emitted without exposure to the toxicant). A 10% of the Microtox Osmotic Adjusting Solution must be added to all samples to provide osmotic protection to test organisms.

6.3.4. *Daphnia Magna* Toxicity Assay

In this acute toxicity test, the biological end point is the immobilisation of the test organisms caused by a suspected toxic aqueous sample. The assay has been standardised (OECD, 2004; ISO 6341:1996), and it is used in the routine control of aquatic toxicity assessment of effluents and in environmental safety evaluation of chemical substances (Barata et al., 2006), and in mechanistic studies concerning aquatic toxicology (Damásio et al., 2007, 2008).

In this assay a group of young crustaceans of the species *Daphnia magna* are exposed to the aqueous sample during a 48-hour period. The number of immobile individuals after 24 hours and 48 hours of exposure is recorded and compared with those in control samples. From these results the concentration causing immobilisation to the 50% of the daphnia population is calculated (EC50). The test organisms used are young *Daphnia magna* (*cladocera, crustacea*), at least of second generation, obtained from acyclic parthenogenesis in laboratory culture conditions and under 24 hours old. Daphnias are not fed during the assay. The assay is run at temperature 20 ºC (± 2 ºC), and under darkness.

In the experimental procedure, a series of at least five concentrations of the test sample and one control were prepared in four replicates. The volume of each test vessel was 10 ml. Five individuals were added to each one of the test vessels, making a total of 20 individuals exposed to each test concentrations. When a solvent had to be used to dissolve the sample (i.e. 0.2% DMSO), a series of control vessels containing the dilution medium had to be added to the test. At the end of the exposure period (24h and/or 48h) the number of mobile *daphnia* in each vessel was counted. The *daphnia* unable to move after a slight agitation of the container were considered immobile, even if they were able to move their antennae. The relationship between test concentration and percentage of mobility was analysed by appropriate statistical methods (e.g. probit analysis) to calculate the concentration causing immobilisation to 50% of the populations (EC50). The measurements were made in triplicate.

6.4. Results and Discussions

Inhibition curves and EC50 values were obtained for a selection of compounds commonly found in European rivers and drinking waters through the use of TOXcontrol™, an automatic on-line biosensor based on the measurement of the inhibition of luminescence by bacteria. The repeatability of the measurements was evaluated by doing experiments in triplicate (coefficient of variation is reported in EC50 values and deviation is graphically shown when representing inhibition curves).

In the first series of experiments, the luminescence inhibition curves were obtained with TOXcontrol™ at 15 min and 30 min. Standard solutions and working solutions (automatically prepared by TOXcontrol ™) are presented in Table 6.1. A selection of resulting inhibition curves is represented in Figure6.2. No results are shown for 30 min exposure of diazinon, propanil and MCPA to the luminescent bacteria. In these cases, problems related to the low stability of standard solutions led to non-reliable results that were discarded for their inclusion in this study.

Table 6.1. Concentration of standard solutions for each analyte and concentration range of working solutions (automatically prepared by TOXcontrol ®)

Compound	Standard solution (mgL-1)	Concentration range (mgL^{-1})	
		Minimum	Maximum
Nonylphenol	1	0,004	0,064
Triclosan	5	0,02	0,20
Terbuthylazine	10	0,32	4,50
Dimethoate	200	0,8	12,8
Diclofenac	1000	4	32
SDBS	1000	8	128
Diazinon	5000	20	320
Propanil	60	7,7	21,2
MCPA	60	3,8	27,0
Cu (II)	318	1	39
Ni (II)	1858	119	476
Cr (III)	10000	40	320
Fe (III)	624	20	80
Pb (II)	1102	18	141

The same organic compounds were tested in an interlaboratory exercise to compare results obtained using TOXcontrol™ and standardised methodologies based on Microtox™ and *D. magna* tests. Table 6.2 shows the EC50 values and coefficient of variation (CV) for the several organic compounds tested with TOXcontrol™ and Microtox™. A collection of results from literature is also provided for comparison. In Table 6.3 the results from *Daphnia magna* tests are included. EC50 values were in agreement with the ones that have been calculated using standardised methodologies (Microtox™ and *D.magna* tests).

Not many results have been reported in the literature for the selected organic compounds and variability can be found between reported values, so it is not easy to establish a comparison. The values obtained for *V. fischeri* using TOXcontrol™ are in general in good agreement with those obtained with Microtox™ and those found in the literature (Table 6.2). Higher differences when comparing the two systems in the framework of this study can be observed, specially at nonylphenol EC50 values (0.03 mgL^{-1} for TOXcontrol™ compared to 54.30 mgL^{-1} for Microtox™, although the literature supports TOXcontrol™ values), propanil (1594 mgL^{-1} for TOXcontrol™ compared to 21.71 mgL^{-1} for Microtox™) and MCPA (3311 mgL^{-1} for TOXcontrol™ compared to 26.10 mgL^{-1} for Microtox™, the literature found the value between these).

Additionally, selected compounds were tested using a different technique based on *D. magna*. Although based on different organisms, it was considered useful to obtain new EC50 values and a comparison was performed to see the correlation between techniques (Table 6.3). Values presenting higher differences between different technologies relate to diazinon presenting values for *D. magna* two orders of magnitude below *V. fischeri* and nonylphenol where differences could reach 4 orders of magnitude between results obtained using TOXcontrol™ and *D. magna.*

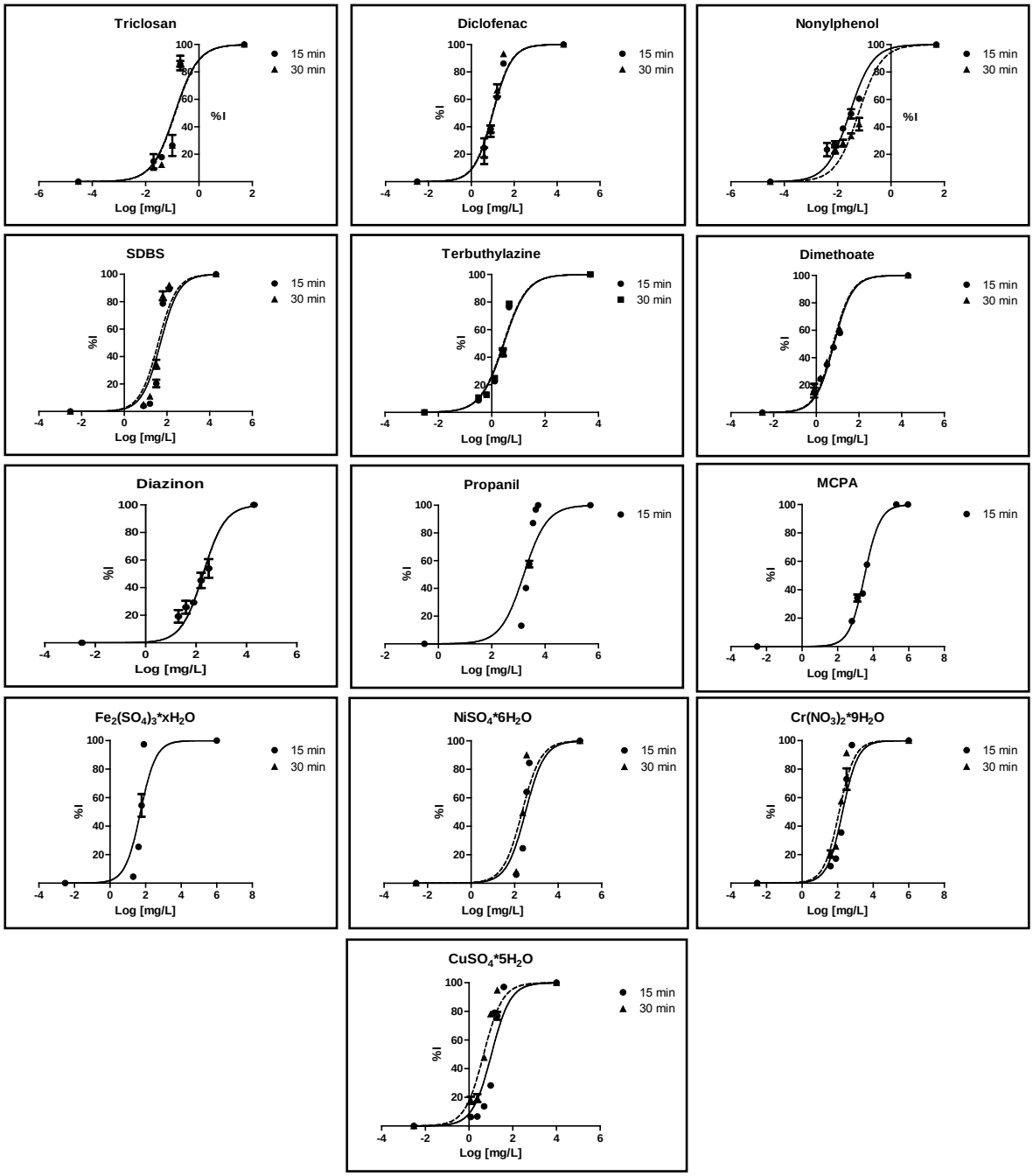

Figure 6.2 Inhibition curves for triclosan, sodium diclofenac, nonylphenol, SDBS, terbuthylazine, dimethoate, copper (II), nickel (II), iron (III) and chromium (III) at 15 and 30 min exposure time and diazinon, propanil, MCPA and iron (III) at 15 min exposure time.

In a new experiment, a second series of tests to determine the toxicity of aqueous solutions of metal compounds with the TOXcontrol™ system was performed. The percentage of inhibition was calculated from the results obtained using metal solutions. The EC50 values at 15 minutes and 30 minutes for copper (II), nickel (II), chromium (III) and iron (III) were calculated (Table 6.2). Standard solutions and working solutions (automatically prepared by TOXcontrol™) are presented in Table 6.1.

Inhibition curves for copper, nickel, chromium and iron are presented in Figure 6.2. The curve for lead (II) was not obtained due to the high variability of values. Even though the exact EC50 value cannot be represented, the approximate toxicological range can be estimated at 70-110 mgL^{-1} (15 min incubation time) and 80-130 mgL^{-1} (30 min incubation time). For the analysis of metal compounds, variations found between values reported in the literature are significant. The methodologies main differences are attributable to the salt used to prepare the solution and the pH adjustment, which is performed in some of the studies (Dutka and Kwan, 1981; Greene et al., 1985).

Table 6.3 Value calculation of EC50 for organic compounds with *D. magna* (exposure time 24 and 48 h)

Compounds	EC50 (mgL^{-1}) t=24h	Range	EC50 (mgL^{-1}) t=48h	Range
Nonylphenol	134	104-172	98	78-123
Triclosan	0.073	0.062-0.086	0.052	0.045-0.060
Terbuthylazine	0.100	0.082-0.159	0.072	0.058-0.097
Dimethoate	2.5	1.8-3.5	1.3	0.4-2.6
Diclofenac	118	-	99	81-123
SDBS (LAS)	23	19-27	15	-
Diazinon	2.5	1.8-3.5	1.3	0.4-2.6
Propanil	14	9-22	3.5	2.2-5.5
MCPA	136	115-163	77	63-95

Table 6.2. Calculation of EC50 (mgL^{-1}) using TOXcontrol® (at 15 and 30 min) and coefficient of variation (CV, N=3) for selected compounds and comparison with values obtained with Microtox® (15 min) and other values reported from literature (min. average, maximum)

Compounds	TOXcontrol ®				Microtox ®		Literature values EC50 (mgL^{-1})			References
	15mEC50	SD	30mEC50	SD	15mEC50	CV (%)	t =5 min	t =15 min	t =30 min	
Nonylphenol	0.03	12.0	0.06	5.4	54.30	24.3		0.36-0.38-0.40		(Farré et al., 2001; Farré and Barceló, 2003)
Triclosan	0.13	6.8	0.13	3.9	0.67	7.1		0.28		(Farré et al., 2008)
Terbuthylazine	2.88	1.5	2.74	1.8	2.04	2.5				
Dimethoate	6.80	2.5	6.20	2.0	0.80	20.7		55	0.9	(Trajkovska et al., 2009;Köck et al., 2010)
Diclofenac	10.26	4.3	9.82	5.5	10.50	1.4		13.7-13.7-13.7	11.4	(Farré et al., 2001; Farré and Barceló, 2003; Ferrari et al., 2003)
SDBS (LAS)	50.04	2.0	39.12	3.0	7.72	6.4		144	14.3	(Gutiérrez et al., 2002; Farré and Barceló, 2003)
Diazinon	192.70	7.2	-	-	240.80	10.4	1.7-26.4-83.74	74.58-79.29-84	74.58-79.29-84	(Chang et al., 1981; Curtis et al., 1982;Somasundaram et al., 1990; Ruiz et al., 1997; Köck et al., 2010; Somasundaram et al., 1990; Ruiz et al., 1997; Köck et al., 2010)
Propanil	1594	2.0	-	-	21.71	6.0	28.8-34.0-35.13	23.9-26.0-28.15		(Kaiser et al., 1994; Ruiz et al., 1997)
MCPA	3311	2.0	-	-	26.10	8.2		248		(Vismara and Garavaglia, 1997)
Copper (II)	10.61	1.9	4.68	3.3			0.72-1.91-6.35	0.102-47.78-580	0.16-12.12-36.0	(Kaiser and Devillers, 1994;McFeters et al., 1983; Qureshi et al., 1984;Tarkpea et al., 1986;Codina et al., 1993;Sillanpää and Oikari, 1996; McCloskey et al., 1996;Vasseur et al., 1988; Codina et al., 2000; Cho et al., 2004; Guéguen et al., 2004; Fulladosa et al., 2005; Rosen et al., 2008)
Nickel (II)	317.2	0.8	221.5	0.9			917-6517-22900	0.13-135-256	42.2	(McFeters et al., 1983; Qureshi et al., 1984; Codina et al., 1993; J Villaescusa et al., 1998)
Chromium (III)	190.4	3.6	123.0	2.2			10.7	15.3	16	(Qureshi et al., 1984)
Iron (III)	52.08	5.4	-	-				16.5-19.5-22.4	13.1	(Sillanpää and Oikari, 1996; Cho et al., 2004)
Lead (II)	70-110	-	80-130	-			2.56	0.12-33.1-237	0.31	(J Villaescusa et al., 1998; McCloskey et al., 1996;Sillanpää and Oikari, 1996; J Villaescusa et al., 1998; Tchounwou and Reed, 1999; Guéguen et al., 2004; Fulladosa et al., 2005; Rosen et al., 2008)

6.5. Conclusions

The results obtained are in the same order of magnitude as those reported in the literature in most cases. It should be highlighted that results from toxicity experiments are dependent on the conditions in which the test is performed. Potential sources of variability could have their origin in the bacteria (preservation, reconstitution procedure, etc.); in the sample (preparation of standard solutions, pH, etc.) and in the experimental procedure (sample handling, deviations in volume delivery, instrumental error, calculation method, etc.). Due to this fact, values obtained from the toxicity test of the same compound can differ depending on the study.

The results presented show that the methodology and the TOXcontrol^{TM} system being validated is accurate and reproducible enough to enable this system to be used as an on-line automatic alert system to detect abnormal concentrations of toxic compounds in surface waters, discharge effluents or drinking waters. The main disadvantage of the use of the instrument as a routine monitor of the water quality concerns the sensitivity of the luminescent bacteria to react when exposed to low levels of toxicants. EC50 values determined for a variety of potential contaminants are above the reported levels of target analytes being currently measured in natural water bodies and drinking water. To solve this problem, the use of already developed on-line pre-concentration modules coupled to the biomonitor system to increase sensitivity could be studied.

Additionally, there is another difficulty when applying this to drinking waters. If some disinfectant has been added and it remains in the sample (e.g. free chlorine), toxicity values will respond to the disinfectant, thereby hiding the real effect of the toxic compounds.

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7

Integration of Ultraviolet-Visible spectral and physicochemical data in chemometrics analysis for improved discrimination of water sources and blends. Application to the complex drinking water distribution network of Barcelona

7. Integration of Ultraviolet-Visiblespectral and physicochemical data in chemometrics analysis for improved discrimination of water sources and blends. Application to the complex drinking water distribution network of Barcelona

7.1. Abstract

The Barcelona Metropolitan Area (BMA) drinking water distribution system is supplied with water from different sources and treated by different technologies. Different blending options occur along the network to homogenise water quality, both chemically and organoleptically. An appropriate technology able to recognise the water source and blends in real time along the network is crucial for the global system management. This paper presents a principal component analysis (PCA) methodology able to discriminate samples with respect to their original source and blends by using UV-Vis data from a spectrophotometric probe and a small number of physicochemical paremetres.

The study began with PCA of 37 physicochemical paremetres obtained through standard laboratory procedures in order to distinguish among sources and blends. Taking a step further, the study investigated the possibility of discriminating the same sources and blends using only UV-Vis fingerprints obtained by a spectrophotometric probe. The discrimination capacity of PCA on UV-Vis data was slightly improved by adding three additional physicochemical paremetres: conductivity, fluoride and boron concentrations. In general, the new model was able to distinguish the two main water origins of the BMA – the Llobregat and Ter Rivers. The contribution of desalinated sea water and groundwater was also distinguished in the blends containing river water. Moreover, the influence of the water sources and blending on the occurrence and speciation of different trihalomethanes (THMs) alongside the BMA was evaluated.

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7.2. Introduction

In recent decades, a significant number of large urban areas, e.g. the Barcelona Metropolitan Area (BMA), which has drinking water supply networks based on surface water resources, experienced a reduction of its water quality and quantity at source due to anthropogenic and environmental pressures(González et al., 2012). Consequently, new infrastructures need to be implemented to improve the quality of drinking water and to ensure the availability of resources in the long term. The current solutions that have been implemented are based on the integration of two approaches. The first is based on the use of membrane-based technology in drinking water treatment plants (DWTPs) (e.g. reverse osmosis, nanofiltration and reverse electrodialysis) to reduce the salinity levels and to remove both organic and inorganic micropollutants; or, when surface and groundwater resources are scarce, the use of seawater desalination technologies. The second relies on the construction of new distribution network interconnections to enforce the blending of the different water sources in order to standardise aesthetic properties and assure the availability of drinking water in all the area covered by the distribution network (Valero and Arbós, 2010).

In the case of large drinking water supply networks delivering water from different sources (e.g. BMA manages up to five different origins), the main changes in the quality of the supplied water in one specific site are caused by water blending operations. Few studies have been published based on the particularities of the blending operations and their impact on water quality. Most of them have dealt with the introduction of desalinated water into a network of water with conventional origins. Examples can be found in Barcelona (Spain) (García et al., 2015; Raich-Montiu et al., 2014), South Australia (Van Leeuwen et al., 2009), San Diego (USA) (Erdal et al., 2013) and Cyprus (Philippou, 2015). Changes in the mixing in the combination of waters coming from different sources produce chemical instability and processes such as precipitation or deposition of carbonates (Lahav et al., 2009) and consequently corrosion of pipes may take place. Several pilot studies have been conducted in order to investigate the impacts of blending waters from different sources on the quality of distributed water with an emphasis on metal release (Taylor et al., 2006). For example, the effects of chlorides and sulphates on lead release from pipes have been proved to be promoted (Tang et al., 2006). Knowledge about water source apportionments at a specific location of the water distribution system is a topic of interest for a better understanding of the network, the detection of possible origins in leak accidents and to establish legal property rights.

Previous studies have tried to predict water origins by testing hydraulic models based on tracers, like conductivity. This approach has been used in Riga, Latvia. Six different water sources, having different electric conductivity values, are used to supply drinking water to the city. The measurement of the tracer was hard to predict when a proper mixing of water took place (Rubulis et al., 2011).

Other studies that have been conducted with traces were addressed to measure water quality based on the decay of paremetres like chlorine, like the one performed in Shanghai Pudong, China (Shu et al., 2010). Although chlorine is used as an indicator of water age, it cannot be used as a tracer of the origin of water as it is added after the intake during treatment processes and transport.

The quality of the produced water is mainly dependent on its natural composition at source, which changes seasonably, and on the operating treatment technologies (Sharp et al., 2006). The main contributors are the content of total dissolved salts, especially in terms of aesthetic properties, and the contribution of the total dissolved organic matter (Spellman, 2007). The characteristics of the natural organic matter (NOM), as a complex mixture of different compounds affected by factors such as vegetation and soil composition, determines the formation of disinfection by-products along the water treatment steps. The complex nature of organic matter makes it difficult to identify both the nature and the concentration of the different compounds (Croué, 2004). Ultraviolet-Visible (UV-Vis) measures have been proposed as a fast technology able to determine the fingerprint of NOM in drinking water and monitor the changes along water treatment systems (Thomas and Burgess, 2007).

The use of the UV-Vis fingerprinting of NOM in natural waters has been proposed as a new additional source of information bringing a clear advantage for a better assessment of general changes in water quality instead of searching for the occurrence of specific contaminants. In this context, the use of UV-Vis fingerprint, by using spectrophotometric probes, has been proposed as a useful strategy to cover a much broader range of potential threats, such as the intrusion of chemical or microbiological constituents in the network (Noij and Bobeldijk, 2003). These in-line/on-line paremetres must show a quick response to potential quality changes, which implies the need for real-time measurements. Spectral data and their evolution over time provide very rich information and an overall picture about water quality changes and, therefore, the possibility to detect changes not recorded by conventional single contaminant analysis (Langergraber et al., 2004). However, evaluating large amounts of data with different time dimensions and identifying the abnormal changes in water quality requires sophisticated analysing tools (Mustonen et al., 2008).

In the case of the BMA, a previous study by Platikanov et al. (2011) proposed a methodology based on the use of UV measurements (190–230 nm) and multivariate data analysis using the Partial Least Squares (PLS) method was developed. The methodology was able to determine the relative amounts of the two main river water sources, the Llobregat and Ter Rivers, in tap samples in the Barcelona area when analysed using an UV-Vis spectrophotometer in the laboratory. Additionally, artificial samples prepared by mixing waters from five DWTPs, including the use of membrane filtration systems, needed the combined use of some other paremetres, like boron, to help to discern the original water sources.

Due to the European Unionregulations, an exhaustive characterisation of water quality is performed by collecting samples from different points in this large network and analysing them at routine control laboratories. Nowadays, more than 80 paremetres are monitored routinely in the BMA water supply network to ensure the safety of the population according to the ISO 22000 certification. The values obtained in these analyses contain valuable information, which can be used for operational purposes if properly handled. The main constraints of this monitoring system are related to the high cost of performing these analyses, and the inability of a fast response when an alteration of the water quality occurs. Therefore, improved monitoring systems based on real-time measures are still needed. The ideal situation would be to design a network of sensors based on optimum placement models able to show water quality variability with the minimum of recorded paremetres (Bazargan-Lari, 2014).

This study goes beyond the one performed by Platikanov et al. (2011) as it introduces the use of a UV-Vis absorbance spectrophotometric probe able to be operated in-line. Identification of the spectra of water samples from different locations in the drinking water supply network and from different seasons of the year has allowed a more comprehensive picture of water characteristics and the determination of water blends from different origins. In a recent study, Raich-Montiu et al. (2014) demonstrated the improvement of the aesthetic properties of the distributed drinking water in the Barcelona Metropolitan Area when particular blending options are favoured. Therefore, monitoring tools to determine the blending ratios will contribute to improve the daily operations of water distribution systems.

7.3. Materials and methods

A description of the case study and sampling procedure of the water samples; the technique used to measure water quality; and the statistics methodology applied for obtaining the information; are included in this section. Barcelona Metropolitan Area (BMA) has been selected, as it is a complex system in terms of water blends and qualities, the availability of restricted data, and the fact that the water production and distribution is following the quality standards of the ISO22000.

7.3.1. Barcelona Metropolitan Area (BMA): site description

BMA covers 635 km^2 and a population of 4.5 million inhabitants. Around 85% of the total drinking water supplied has its origin in surface waters (Ter and Llobregat Rivers) while the remaining 15% comes from groundwater resources.

In order to adapt the drinking water system to the new European legislation requirements, the DWTPs in Barcelona have conducted new infrastructures in recent years. Additionally, the severe drought that occurred in 2008 encouraged the use of new alternative resources. A quite complex combination of water sources and treatments are currently used in the Barcelona distribution system: a) Abrera DWTP, with a new treatment line incorporating reverse electrodialysis, and Sant Joan Despí DWTP, including an additional step of ultrafiltration and low pressure reverse osmosis, both located in the lower-middle course of the Llobregat River, supply approximately 40% of the drinking water to the Barcelona Metropolitan Area; b) Cardedeu DWTP treating Ter River water with a classical process including coagulation, flocculation and activated carbon filtration; c) El Prat Seawater Reverse Osmosis Desalination Plant (SWRO); and d) various groundwater wells at the Llobregat and the Besòs Rivers basins(García et al., 2015). A diagram representing all these water sources is shown in Figure 7.1.

The integration of both membranes processes in Abrera DWTP and Sant Joan Despí DWTP have helped to reduce the salinity in the distributed water (the Llobregat River contains approximately 0.9 gL^{-1} of total dissolved solids) and the reduction of precursors of disinfection by-products (e.g. ammonium, bromide, iodide and NOM). Both plants have achieved a reduction in the formation of trihalomethanes (THMs) in drinking water to levels below 100 μgL^{-1} as required by the Drinking Water Directive (98/83/EC)(Valero and Arbós, 2010). Ter River water is treated without any membrane technology since the level of salinity is slightly lower (with a total dissolved solids level of approximately 0.35 gL^{-1}).

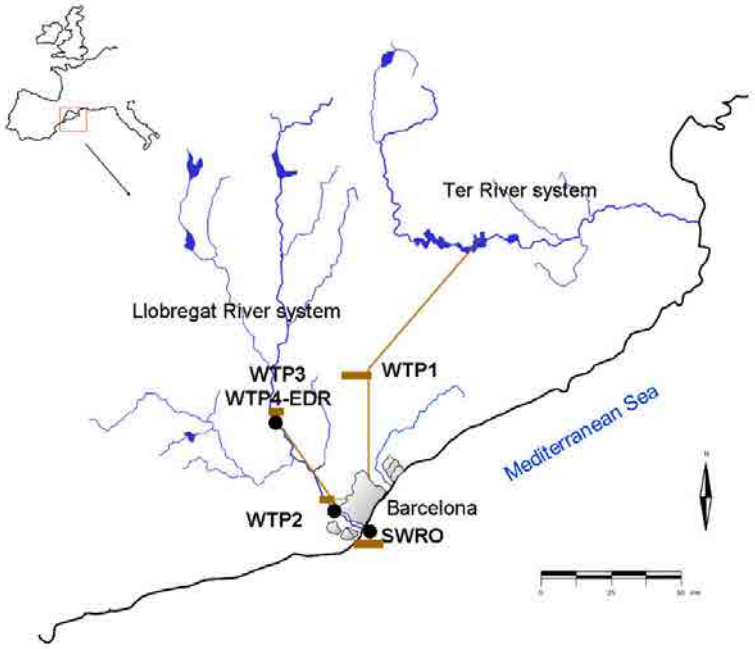

Figure 7.1. Diagram of different water supplies in Barcelona area: sources and treatment plants distribution. WTP1: Cardedeu DWTP; WTP2: Sant Joan Despí DWTP; WTP3: Abrera DWTP classical treatment; WTP4-EDR: Abrera DWTP plus reversible electrodialysis; SWRO: El Prat Seawater Reverse Osmosis plant

7.3.2. Sampling

Aigües de Barcelona, which is the water utility operating the drinking water distribution system in Barcelona, contributed to the study by providing a total of 191 drinking water samples over one year (April 2012-March 2013). The samples were collected from various locations in the dense BMA drinking water supply system during several seasons in order to cover the maximum variability. Samples with a contribution from one single source were also included in order to have a characterisation of these water origins.

The samples from every location were examined in situ for three paremetres (free chlorine, total chlorine and temperature). In addition, data about 82 physicochemical paremetres were assessed at the Aigües de Barcelona laboratory. After the analysis and the compilation of data, the paremetres which were permanently below the limit of detection (LOD) for most of the observed samples or did not show variability were discarded from the chemometric analysis. As a result of this data filtering, 37 physicochemical paremetres were selected. Table 7.1 presents the investigated physicochemical paremetres and their descriptive statistics.

7.3.3. UV-Vis absorbance spectrophotometric probe

All samples were recirculated in a closed loop through a UV-Vis absorbance spectrophotometric probe (spectro::lyser^{TM}, s::can, Austria) with the help of a pump. The probe can be installed and operated remotely but, for the purposes of this study, it was placed in the laboratory in order to be able to analyse samples from multiple locations. Each sample was measured 10 times in order to obtain an average of the water fingerprint. Data were exported into an Excel file for their statistical treatment.

7.3.4. Chemometric tools for data treatment.

Experimental data containing 37 physicochemical paremetres and UV-Vis spectra (absorbance values at 101 different wavelengths) for the 191 samples were arranged in two single data matrices, X1 [191, 37] and X2 [191,101]. A third, row-wise augmented data matrix X3 [X2 plus conductivity, fluoride and boron paremetres] was built to investigate the advantages of using these data.

Preliminary exploratory data analysis was performed using univariate descriptive statistics. Each physicochemical paremetre and the UV-Vis fingerprint were plotted individually for the different

measurements performed during the whole investigation period. Histograms and box plots were analysed for trends and outliers. Pair-wise correlations among all variables were also evaluated.

Table 7.1. Averages, maximum and minimum values; and the total number of samples with reported values above the detection limit for the selected 37 physicochemical paremetres analysed in the water samples

Paremetres	Units	Values > LOD	Average*	Maximum	Minimum
Free chlorine*	mgL^{-1}	190	0.72	1.13	0.15
Total chlorine*	mgL^{-1}	191	0.88	1.31	0.2
Temperature*	ºC	191	17.4	29.8	8
Fluoride	mgL^{-1}	123	0.13	0.28	0.1
Nitrates	mgL^{-1}	190	7.6	47	0.8
Bromoform	μgL^{-1}	145	23	70	1
Chlorodibromomethane	μgL^{-1}	188	7	64	1
Chloroform	μgL^{-1}	100	20.7	49	1.1
Dichloromethane	μgL^{-1}	155	6.15	19	0.5
Trihalomethanes	μgL^{-1}	188	41.3	99	5.9
Aluminium	μgL^{-1}	166	57	185	25
Barium	μgL^{-1}	177	32.5	152	0.9
Boron	μgL^{-1}	168	151	828	26
Calcium	mgL^{-1}	191	73	142	9
Strontium	mgL^{-1}	174	0.8	1.85	0.1
Iron	μgL^{-1}	147	17	151	5
Phosphorus	μgL^{-1}	30	25	37	20
Lithium	μgL^{-1}	75	15	22	10
Magnesium	mgL^{-1}	187	17	47	2
Nickel	μgL^{-1}	64	7	18	5
Potassium	mgL^{-1}	129	16	34	5
Silicon	mgL^{-1}	170	1.7	8.2	0.5
Sodium	mgL^{-1}	187	70	179	15
TOC	mgL^{-1}	143	1.9	11.9	1
Free chlorine	mgL^{-1}	183	0.86	51	0.15
Chloride	mgL^{-1}	189	118	299	24.9
Conductivity (20ºC)	μScm^{-1}	190	765	1899	58
Ind. Langelier	Langelier units	191	-0.043	0.782	-2.965
pH	pH units	191	7.4	8,0	4.7
Sulphates	mgL^{-1}	189	85	205	8
Turbidity	NFU	86	0.4	1.6	0.2
Alkalinity	mgL^{-1} CaCO3	191	157	288	24.5
Bicarbonates	mgL^{-1} HCO3	179	192	351	85.8
Bromide	mgL^{-1}	45	0.17	0.36	0.1
Chlorates	μgL^{-1}	190	492	2440	42
Chlorites	μgL^{-1}	61	99	262	20
Hardness	mgL^{-1} CaCO3	191	252	516	18

In order to investigate how much information explained by physicochemical data overlaps with the information in the UV-Vis measurements, the modified RV coefficient was calculated (Smilde et al., 2008). The modified RV coefficient is a rotation invariant measure of the similarity between two cross-product matrices with values in the range of [-1,1]. The closer the RV coefficient is to 1, the more similar the two cross-product matrices are. The interpretation of modified RV is similar to the interpretation of the Pearson's correlation coefficient (Stanimirova et al., 2011).

The Principal Component Analysis (PCA) technique was applied to data matrices X1, X2, X3. This method extracts information about the latent structures of the data set. It transforms a large number of correlated original data (in our case, water quality paremetres and UV-Vis spectral data) into a reduced number of uncorrelated, orthogonal variables explaining maximum variance, called principal components (PCs) (Jolliffe, 2002). The samples from different locations of the investigated area were projected onto these principal components, giving sample scores. Plots of these sample scores and of the corresponding loading plots of physicochemical paremetres and spectra allow the investigation of the main sources of data variance. The main advantage of using PCA is the reduction of the dimensionality of the problem (number of paremetres or spectral wavelengths) retaining most of the original variability in the experimental data but filtering out noise and other minor irrelevant sources of data variance. Therefore, PCA allows for a more simple interpretation of variance sources of the investigated data set.

7.4. Results and discussions

The main goal of this study was to build up a multivariate model able to describe and distinguish different water sources and blends in the drinking water of the BMA obtained as a function of their physicochemical paremetres (obtained at the laboratory) and of the measured UV-Vis spectra. The PCA model based on UV-Vis spectral data will be compared with the PCA model built on the laboratory physicochemical data.

Firstly, physicochemical paremetres data matrix, X1, and UV-Vis fingerprints matrix, X2, were analysed separately. A comparative study was performed to test the similarity between both data sets and to identify the amount of information that UV-Vis real-time monitoring could provide compared to laboratory routine analysis. Additionally, in order to improve results, the analysis of the combination of UV-Vis data with a selection of physicochemical paremetres, in matrix X3, was carried out.

7.4.1. Descriptive statistics and preliminary inspection of the physicochemical and spectral data matrices

A total number of 37 paremetres measured in the laboratory were used for statistical analysis. Table 7.1 shows variations of the water quality paremetres. Averages, maximum values, minimum values, the total number of samples and the number of samples with reported values above LOD are included.

Analysing the information contained in Table 7.1, it can be predicted that paremetres having a higher variability in their values would contribute to a greater degree to the classification of the water samples.

This is the case with paremetres like conductivity (58 to 1899 μScm^{-1}), or the concentration of boron (26 to 828 μgL^{-1}), sodium (15 to 179 mgL^{-1}) and calcium (9 to 142 mgL^{-1}). It can be seen that some other paremetres fluctuate within a narrower range. The concentration of these paremetres is altered as the water is treated in the DWTPs and their values tend to homogenise. This is the case with chlorine concentration (0.2 to 1.31 mgL^{-1} of total free chlorine measured *in situ*) or total organic carbon (TOC) levels (1 to 12 mgL^{-1}). Due to the treatments performed in the DWTPs, different water origins will influence the nature of organic matter in drinking water more qualitatively than quantitatively.

Pairwise correlation coefficients between the 37 physicochemical paremetres were either positive or negative (see Figure 7.2). In the central part of the correlation map, strong positive correlations (intense red colours) can be seen between lump paremetres such as conductivity, hardness and alkalinity with cations like sulphates, calcium, magnesium, sodium or chlorine as expected. These paremetres are characteristic of natural water mineralisation. Negative correlations (intense blue colours) were found between paremetres in this first group and other paremetres resulting from the DWTP operation such as chlorine dioxide, TOC or disinfection by-products, which formed another cluster. Other paremetres such as turbidity, temperature and free residual chlorine did not show any positive or negative correlation. The temperature shows seasonal variations and therefore it affects all samples.

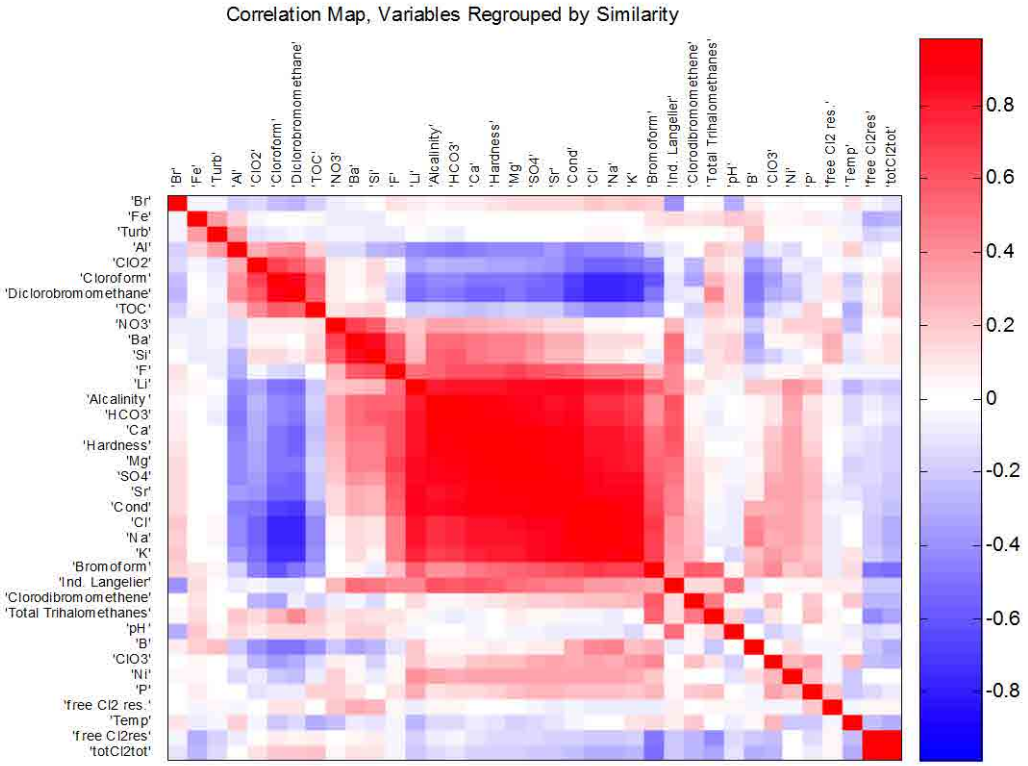

Figure 7.2. Correlation map among the 37 physicochemical paremetres measured in 191 water samples

Some other paremetres of interest, such as THMs, can be linked to their precursors. The content of chloroform and dichlorobromomethane show a positive correlation with the content of TOC and of chlorine dioxide, and a negative correlation with waters with high salts content. In contrast, dibromochloromethane and bromoform show the opposite trend. This is interpreted as the formation of different types of THMs depending on the water origin of the sample. Water, coming from the Llobregat River and to a lesser degree from the SWRO plant, leads to a trend of brominated THMs formation, while water coming from the Ter River tends to form chlorinated THMs.

The modified RV coefficient (Smilde et al., 2008) for the two pre-treated data sets in this work, X1 and X2, was found to be 0.85, indicating that they have a large amount of overlapping information. This relatively high correlation coefficient proves that the information contained in the spectra is comparable to the information provided by more expensive laboratory analyses of physicochemical paremetres.

Figure 7.3 shows the fingerprint of three pure water samples from different sources. These fingerprints depend on the composition of organic matter in the analysed water. Changes can be seen in the absolute values recorded at the same wavelength for different samples and in the ratios between absorbance values at different wavelengths for the same sample. In this case, the water fingerprint for the Cardedeu DWTP sample presents higher absorbance values than the other samples. The lowest values relate to the Besòs DWTP sample, where groundwater is treated by reverse osmosis giving a low content of organic matter. Additionally, the fingerprint of the Cardedeu DWTP sample shows an absorbance peak at 202 nm and a shoulder at 215 nm. These trends confirm the different nature of organic matter presented in the water samples analysed in this work.

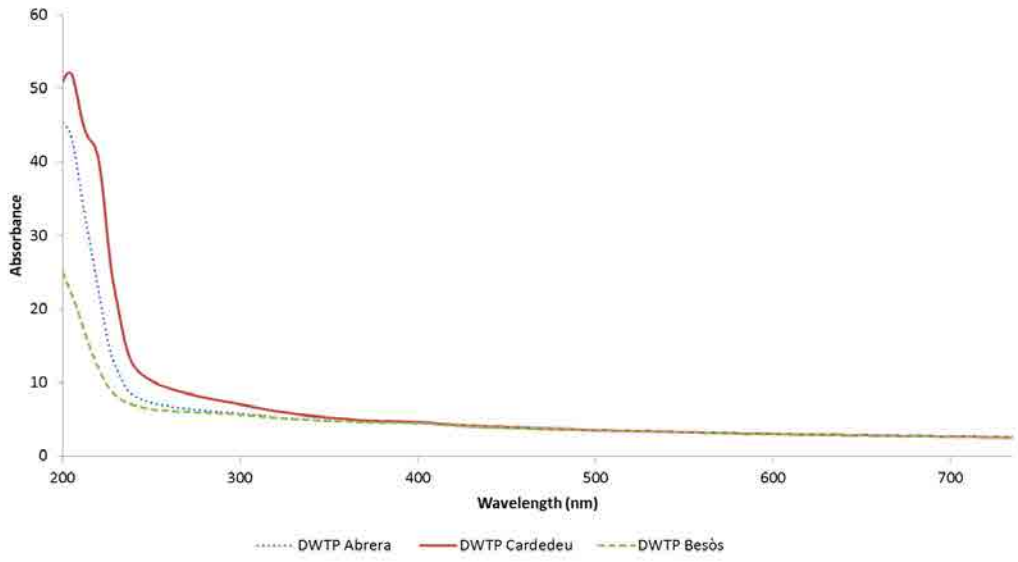

Figure 7.3. UV-Vis spectra of three water samples (April 2012) from different origins (DWTP Abrera, DWTP Cardedeu and DWTP Besòs)

7.4.2. PCA analysis of the physicochemical data

Before PCA, data from physicochemical paremetres were autoscaled (column centering and scaling to a unit standard deviation) (Massart et al., 1998).

PCA of X1 matrix gave 9 components (PC1:37.3%, PC2:12.4%, PC3:8.5%, …, PC9:3.0%) with eigenvalues higher than 1, which explained 82% of the total variance of the data. This rather large number of principal components indicates multiple sources of data variance, probably due to the variety of water sources, the different technologies implemented at the DWTPs and the large variety of paremetres (either from natural or from anthropogenic origin).

Figure 7.4(a) shows the scores plot for the projection of samples onto the first two components, PC1 and PC2. These two PCs explain 50% of the data variance. Their scores plot shows that samples with different water blendings have been distinguished very well. For example, samples from the same river as main source are placed in the same region of the scores plot, and samples are grouped in clusters depending on the other water source contributing to the blending. As a general rule, the samples with large positive scores on PC1 contain a large contribution from the Llobregat River while those with strong negative scores on PC1 relate to the Ter River samples.

According to PC2, water samples blended with groundwater are characterised with positive scores on this PC. The groundwater contribution pushes water samples to different zones of the plot depending on the source wells, since groundwater from different origins presents different characteristics. Water samples having high contributions from SWRO are located in the same cluster, irrespective of their river source, with negative scores in PC2.

Figure 7.4(b) shows the PC1-PC2 loadings plot. Paremetres reflecting natural origin and high water mineralisation tend to have positive loadings in both PC1 and PC2. In relation to THMs, bromoform is present with a positive loading on PC1. In contrast, chloroform has a negative loading in PC1. PC2 is dominated by boron, which has the highest negative loading on this PC.

Combining the information obtained from PC1-PC2 score and loading plots, it is possible to conclude that PC1 distinguishes between the two main river water origins (Ter and Llobregat Rivers). On the other hand PC1 also describes quality paremetres showing water mineralisation. Samples containing water from the Llobregat River origin were characterised by higher concentrations of these mineral paremetres than samples from the Ter River origin. Samples from the Ter River water origin were found to include higher concentrations of chlorine and chloroform in contrast to samples from the Llobregat River origin, which

contain the highest bromoform concentrations. This analysis proves that, within a large metropolitan area such as the BMA, THM formation processes can follow different mechanisms due to different water sources with different chemical compositions.

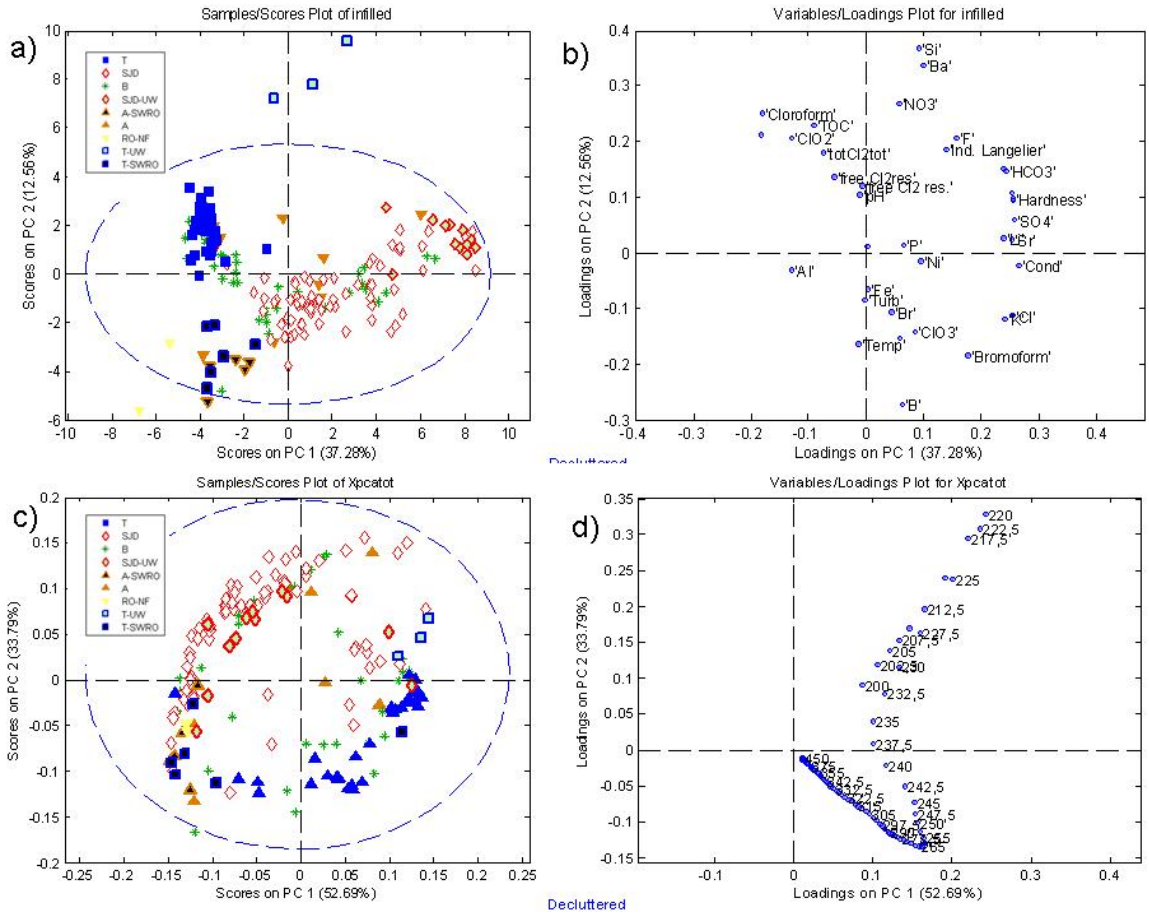

Figure 7.4. (a) PCA scores plot of water samples for 37 paremetres measured in the laboratory and (b) the corresponding loading plot (c) PCA scores plot of UV-Vis spectral data and (d) the corresponding loadings plot. Legend: T, water samples with Ter River origin; T-UW, blended water from Ter River origin with groundwater; T-SWRO, blended water from Ter River origin with desalinated sea water; SJD, water samples from Sant Joan Despí DWTP with Llobregat River origin; SJD-UW, blended water from Sant Joan Despí DWTP (Llobregat River origin) with groundwater; A, water samples from Abrera DWTP with Llobregat River origin; A-SWRO, blended water from Abrera DWTP (Llobregat River origin) with desalinated sea water; RO-NF, water samples from the Besòs River, treated with reverse osmosis and nanofiltration technologies; B, water samples from the water distribution system in the area of Barcelona

Throughout PC2, samples can be characterised according to their blending with water obtained from filtration processes or by addition of groundwater. Boron concentrations were found at relatively high concentrations in all samples blended with desalinated sea water due to its incomplete rejection during the

reverse osmosis filtration in the SWRO plant. On the other hand, river water blended with groundwater showed higher concentrations of minerals such as fluorine or silicon.

This analysis of physicochemical paremetres of water samples (data matrix X_1) has proved that water samples can be distinguished and clustered in the PC1-PC2 plots, summarising the information provided by the 37 physicochemical paremetres and making the interpretation of their changes (variance) easier for a better management of the drinking water system.

7.4.3. PCA analysis of UV-VIS spectral data

Data obtained by UV/VIS spectrophotometry also required some initial pre-treatment. Firstly, data were column min-max scaled (García-Reiriz et al., 2014) so the minimum and the maximum of the transformed spectral absorbance values were comprised between 0 and 1. Secondly, data were mean-centered in all cases to avoid offset effects; and finally, spectral data were normalised to the unit area to enhance possible spectral differences among them. PCA analysis on the pretreated data X2 matrix gave a model with four components (PC1: 57%, PC2: 28%, PC3: 8.6%, PC4: 5.5%), which explained 99% of the total variance.

Figure 7.4(c) shows the PCA scores plot of the two first components, PC1 and PC2, explaining 85% of the total data variance. PC1 is determined mainly by negative scores for samples, either from the Llobregat River or from the Ter River origin, blended with water treated with membranes such as SWRO and Besòs DWTP origins. The strongest positive scores on PC1 were found for samples with the Ter River origin blended with groundwater. In contrast, samples with the same Llobregat River origin but blended with groundwater did not show a significant pattern distribution along this PC1.

PC1-PC2 loadings plot shown in Figure 7.4(d) indicates that in PC1, all spectral bands dominate with positive loadings. Water treated with membranes (SWRO and Besòs DWTP origins) reduced the effect of the river source water, resulting in a decrease of the water spectral intensity. However, the blending with groundwater showed a stronger effect for samples containing the Ter River origin than for samples containing the Llobregat River water origin. In this case, it is PC2, which shows the distribution of samples according to the two main river water sources (Ter River and Llobregat River). Positive loadings on PC2 relate to the Ter River water samples and negative loadings on PC2 to the Llobregat River origin.

Although this study proves that UV-Vis spectra can differentiate waters from different origins, the overall distribution by sources and blends is not as clear as the previous model obtained using the water physicochemical paremetres. However, the possible advantage of using this second strategy is based on the use of a single probe to obtain real-time information about water quality. To make the application

more useful, a combination of UV-Vis spectra and a limited number of physicochemical paremetres is proposed.

7.4.4. PCA of augmented UV-Vis data set with fluoride, conductivity and boron physicochemical paremetres

PCA analysis was applied to the augmented data set X3, which includes UV-VIS data and fluoride, boron and conductivity physicochemical paremetres. The pre-treatment of the augmented data block consisted of column min-max scaling, mean centering and normalisation to the unit area. PCA identified four components with significant values (PC: 36.6%, PC2: 32.0%, PC3: 15.0%, PC4: 7.3%) which explained 90.9% of the total variance of the data.

Figure 7.5(a) shows the PCA scores plot for the two first components, PC1 and PC2, explaining 68.6% of the total data variance.

In the first component, PC1, the samples from Llobregat River were distinguished from the samples with the Ter River origin. The Ter River origin samples were distributed predominantly on the lower right-hand side of the plot with strong positive scores on PC1. In contrast, samples from the Llobregat River were distributed predominantly on the upper left-hand side of the plot with strong negative scores in PC1. PC2 was determined by the blending effect, either mixed with filtered water or with groundwater. River samples from both rivers, blended with groundwater, showed positive scores in PC2. In contrast, samples from both rivers and blended with filtered water were very well clustered on the lower left-hand corner of the plot, with negative scores in this PC.

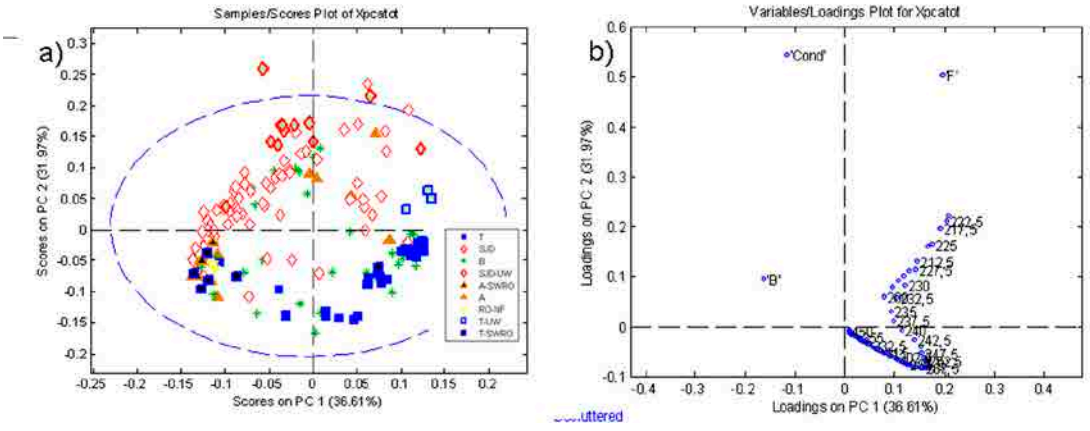

Figure 7.5. (a) PCA scores plot of UV-Vis spectral data plus three paremetres (conductivity, fluoride, boron) and (b) the corresponding loadings plot. For text explanation, please refer to Figure 7.4 caption

In the loadings plot, Figure 7.5(b), the region related to the Ter River origin shows a higher contribution from absorbance values at medium wavelength range (250-280nm). In contrast, the area that was correlated with Llobregat River showed strong absorbance values in the lower wavelength range (210-230nm) plus the paremetre conductivity. Positive loadings on PC2 at the area where groundwater has been identified refer to the concentration of fluoride. In the region of the plot associated with filtered water, negative loadings on PC2 were found for the concentration of boron.

PC1-PC2 scores and loadings plots suggest the importance that a third PC3 could contribute for a better discrimination among water sources, especially between the Ter and Llobregat River samples. Figure 7.6 shows the distribution of samples according to their water type along the PC1, PC2 and PC3 axes. Two different directions in the sample scores distribution on this plot are obtained. The red arrow line (hand written) indicates the distribution of Llobregat samples, starting with samples blended with seawater origin, pure Llobregat River water sample and river water blended with groundwater. On the other side, the blue arrow indicates the sample distribution of water samples from Ter River water.

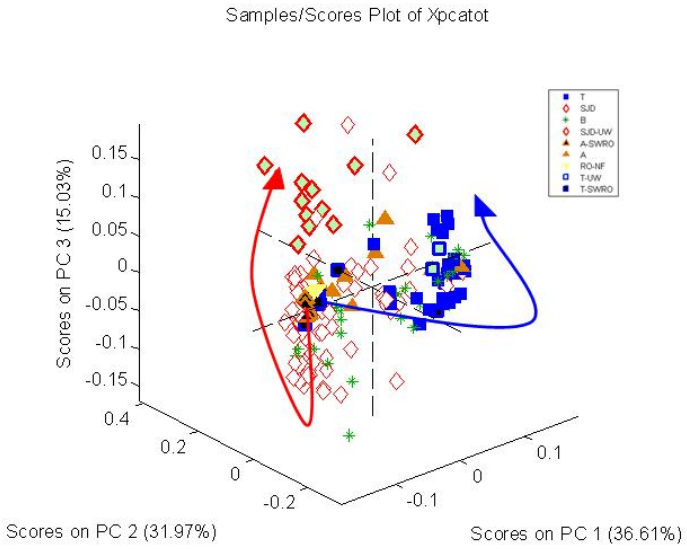

Figure 7.6. PCA scores plot of UV-Vis spectra plus three paremetres (conductivity, fluoride, boron) measured in water samples projected onto the three first principal components PC1, PC2 and PC3. For text explanation, please refer to Figure 7.4 caption

The examination of the results obtained from PCA analysis of the augmented data matrix confirmed and summarised previous findings from the individual PCA of physicochemical and UV absorbance data. The final goal of our investigation, which was to use the spectral data to characterise the water origin, including, if necessary, a limited number of physicochemical data, has been achieved. Based on a previous study (Platikanov et al., 2011), fluoride has been confirmed to contribute significantly to the identification of the presence of groundwater in water blends. Also, the contribution of seawater was able

to be monitored when boron was included as an additional paremetre. In order to reinforce the differentiation between pure Llobregat River and pure Ter River water, conductivity has been proposed as an especially useful paremetre.

7.5. Conclusions

This study has proved the feasibility of predicting the contribution of water sources to the Barcelona drinking water network by applying chemometrics not only to the large amount of data obtained from laboratory analyses but also to the data obtained when on-line measurements (e.g. UV-Vis spectral fingerprint and conductivity) and other off-line measurements (e.g. fluoride and boron) are used simultaneously.

In this study at the BMA, the two main surface water contributors, the Ter River and the Llobregat River, were distinguished by presenting different mineral and organic matter composition. PCA analysis indicated the influence of two different spectral regions, which were characteristic of the two river sources. These two spectral regions are related to their chemical composition, in particular to their different organic matter fractions, thereby making their differentiation feasible.

Additional important information for the water distribution management system can be also obtained from the correlation between water paremetres, such as the possible influence of water blending in the formation of different species of THMs.

The prediction power of UV-Vis spectral data was slightly improved, when physicochemical paremetres that can be measured on-line, such as conductivity (for its discriminating power between different water sources), boron concentration (for its capacity to characterise seawater origin), and fluoride concentration (as a marker of groundwater origin). The classification of water samples according to their source contribution was achieved.

The results of this work confirm that a tool based on on-line/in-line real time measurement of the selected paremetres and the subsequent chemometric analysis can be of great help for the operation of complex drinking distribution systems using water blending.

In terms of sustainability, the work can contribute to reduce the number of chemical analyses performed routinely. The technology proposed for measuring water quality is a reagent free instrument to be installed on-line, without the need of water sampling and transport. In combination with the proper statistic methodology, one single instrument has been proved to offer almost as much information as a list

of individual paremetres. As result, advanced information to the operators can be provided reducing the cost and the ecological footprint of the chemical analysis. Other applications based on the same detection technology can be addressed for future research, like the ability to detect contamination events if proper data treatment is applied.

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8

Ecological screening indicators of stress and risk for the Llobregat River water

8. Ecological screening indicators of stress and risk for the Llobregat River water

8.1. Abstract

The objective of this study is to develop and apply several simple and general indicators for river aquatic ecosystems assessment in order to screen potential chemical stressors. We developed several indicators, based on toxicity (PNEC) and on legislation levels (EQS). All these indicators are ratios that were calculated by using public and private data on the concentrations of a long list of compounds over a period of five years, including metals and organic compounds in the lower part of the Llobregat River basin at the intake of the Drinking Water Treatment Plant. Additionally, new campaigns were executed to increase the information available about the presence of compounds not routinely analysed, such as some other pesticides and pharmaceuticals. In the case of inorganic pollutants, the indicators obtained in this river section showed significant risk especially for zinc, but also for copper, nickel and barium. For organic pollutants, the pesticides terbuthylazine, diazinon, 2-methyl-4-chlorophenoxyacetic (MCPA), and in a few cases, chlorpyrifos and lindane, also showed indexes above the threshold. Among the pharmaceuticals, the antibiotics clarithromycin and ciprofloxacin were the only ones with risk indicators adverse to ecosystems. The specific values of the indexes obtained rely on the quantity and quality of the data available, so their interpretation should take into account that some values can be high due to the use of too conservative toxicological information.

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8.2. Introduction

Degradation of water bodies has been a key issue in Europe in recent years. The Water Framework Directive (WFD, Directive 2000/60/EC) [1] imposes the achievement of good ecological status of water bodies. Environmental objectives should preserve the quality of water bodies beyond its potential uses for industry, agriculture, urban and recreational uses, by integrating preservation of the health of ecosystems, their functioning and structure. This objective should assure the long-term preservation of ecosystems and local biological communities, as well as the elimination of dangerous substances that could pose a risk to human health.

National administrations, such as river basin authorities, should use indexes that could be easily used to give an indication of the good chemical, hydromorphological and biological status of each specific water body according to their local characteristics.

The threshold for this good status should be established to prevent a significant alteration of water bodies. This means biological communities should be healthy and physico-chemical and hydromorphological paremetres must show that no major changes have occurred compared to the base value in their natural state [2].

Indexes for physico-chemical and biological status are relatively easy to implement. Measurements are based on data that can be obtained by analysis, either in the field, in the laboratory or in real time at monitoring stations, or by the identification and counting of species. Concerning specific pollutants, Directive 2008/105/EC [3] establishes Environmental Quality Standards (EQS) for a list of 33 priority substances. These standards have been obtained from toxicological studies that show a clear correlation between chemical and biological responses. The monitoring of these substances implies a high cost in laboratory analyses and the information is not always easy to interpret and aggregate. Additionally, the proposal for amendment of the above mentioned Directive establishes EQS for the biota for some of the legislated compounds. [4]

In order to have a clear view of the pollutants posing major stress at a specific site, it would be very helpful to gather information on the chemical status over a long period of time and normalise concentrations values according to a reference value, thereby giving an indication of their harmful potential. WFD requirements for achieving a good ecological status do not include guidelines on how to select the most appropriate stressor-specific environmental indicators.

Previous studies focused on the task of establishing Water Quality Indexes (WQI) to give an indication of water-body quality beyond the concentration of individual paremetres. These indexes can be based on a

fixed list of paremetres or they can be case dependent, considering specific pollutants according to the most common impacts in every case. The main problem associated with these indexes is the limited range of paremetres to be integrated, which can underestimate the ecological impact. A long list of applications of WQI are found in the literature, applying or customising the most common ones in different countries around the world such as Turkey [5], Iran [6], Chili [7], Zimbabwe [8], Argentina [9], etc.

More advanced studies are conducted to combine bioassessment and modelling techniques, such as the one performed in Denmark [10]. Some of the work to create new indexes also includes application to Geographical Information Systems (GIS) [11] and web-based approaches [12]. Studies about dealing with the uncertainty of environmental risk prediction have been undertaken [13]. Gottardo [14,15] proposed a methodology for Integrated Risk Assessment (IRA) based on a Fuzzy Inference System in order to hierarchically aggregate a set of environmental indicators. Fuzzy logics have been applied in recent years in order to develop risk indicators [16,17].

The study published by Van der Ohe [18] presents a more similar approach to the study presented below as a prioritisation of a list of chemicals is performed, according to a decision tree, for their monitoring based on the information available for 500 organic substances in four European basins.

The idea of establishing a comprehensive index is to provide a unique indicator on water quality for environmental managers. This work on simplicity could be very useful but the information on the impact on single paremetres is lost. This work focuses on giving a simple indicator about the impact on every pollutant that can be found in the Llobregat River waters, considering its effect on aquatic and vertebrate organisms and considering its relation to legislative thresholds, referred as EQS.

8.3. Methodology

8.3.1. Risk indexes determinationmethodology

Legislation has been developed applying the concept of aquatic ecosystem protection and establishing EQS for priority substances [3]. Further biota EQS have been proposed for future amendments [4]. The methodology for deriving these standards is based, among others, on the concepts of Ecological Risk Assessment (ERA) based on PNEC (Predicted Non Effect Concentration) and PEC (Predicted Environmental Concentration) [19].

Taking PEC_j as the concentration of a contaminant j measured in water, a risk indicator of aquatic organisms, $I_{ao,j}$ is defined as follows:

$$I_{ao,j} = \frac{PEC_j}{PNEC_j} \quad (1)$$

$PNEC_j$ is derived from toxicological values in water, basically the NOEC (No Observed Effect Concentration) of crustaceans, algae, and fish, and the correct safety factor (assessment factor, AF).

For priority substances, EQS give a concentration that represents impact on aquatic media, $C_{REF,j}$. In these cases, where the threshold concentration for pollutants is legislated, a similar indicator to the one given in expression (1) could be derived, by replacing the PNEC with the legislative value EQS:

$$I_{am,j} = \frac{PEC_j}{C_{REF\ j}} \quad (2)$$

Where $I_{am,j}$ is an indicator of aquatic impact

The protection of terrestrial vertebrates (mammals and birds) that are predators of aquatic organisms are also part of the aquatic ecosystem and could be assessed by comparing the concentration of contaminants in aquatic organisms (PEC_{food}) with the value of PNEC expressed in the food basis ($PNEC_{food,j}$) [19].

$PEC_{food,j}$ could be expressed by using the transference Bioconcentration Factor (BCF) that measures the ratio concentration of contaminant in small aquatic organisms (considered food) ($PEC_{food,\ j}$) divided by the concentration of contaminant in water (PEC_j). In this way, an indicator of terrestrial vertebrates risk, $I_{tv,j}$ could be obtained with the following expression:

$$I_{tv,j} = \frac{PEC_j}{PNEC_{food,j} / BCF_j} \quad (3)$$

BCF_j values could be obtained from empirical studies or, in case of organic compounds, from correlations with log K_{ow} (Octanol-Water Partition Coefficient).

The above expressions show that, having the concentration of the contaminants in water (PEC_j), the calculation of all the exposed indicators can be performed. For all these indicators, a target value of 1 was taken as the limit of a correct environmental situation.

Every risk assessment process should consider the potential effect of a given substance and its exposure level [20], but certain aspects should be considered when creating and calculating indexes based on the

environmental concentration and exposure of pollutants. On one hand, the use of maximum or average concentrations in a given period of time and the treatment of data below the limit of quantification (LOQ) are needed to be taken into account. On the other hand, there is a tendency to equate the concentration of a non-quantified substance to half the value of LOQ. This strategy could lead to an overestimation of their ecological risk [21].

8.3.2. Case study and data sources

The proposed ratios were calculated using data about concentrations from a list of contaminants in the Llobregat River water. The Llobregat River basin is situated in Catalonia (NE, Spain) and covers a catchment area of about 5000 km2 which is inhabited by more than 3 million people. The Llobregat River is 156.5 km long, but in this study, a specific location was selected close to the mouth of the river, just before the intake of the biggest Drinking Water Treatment Plant (DWTP) supplying water to the Barcelona Metropolitan Area (UTM coordinates X: 420340 Y:4578442). The site was selected for two main reasons. As it is in the lower part of the Llobregat basin, the river receives discharges from urban and industrial wastewater treatment plants, sewer overflows and diffuse pollution from agricultural fields, being the area with the highest impact along the river. According to the Catalan Water Agency (ACA), the administration responsible for preserving water quality in the Llobregat basin, the chemical status of the selected point is regarded as “bad” with a remark on the occurrence of surfactants, pesticides and heavy metals [2]. The location before the DWTP gives information on the potential impact on health for consumers. This location is not only advantageous because of this dual objective of protecting environmental and human health, but also because of the higher intensity of existing monitoring programmes.

The data used for the first part of the study were obtained from two main sources. ACA communicates information on water quality paremetres through its website [22]. Monthly averages were downloaded for the period 2006-2010 for a list of 154 paremetres. Only contaminants showing values above LOQ for the specific location were selected. Lump paremetres showing physico-chemical conditions were discarded in order to focus only on specific pollutants. Selection was also restricted to those contaminants where a toxicological reference value could be obtained. Sociedad General de Aguas de Barcelona (SGAB), in charge of operating the DWTP, also supplied its record of data for 326 paremetres for the same period. The same methodology was executed for the final selection.

After the selection was performed, impact indexes were calculated based on ACA data for the metals barium, nickel and zinc and the organic compounds chlorfenvinphos, chlorpyrifos, fluorides, lindane, simazine and terbuthylazine. In the case of SGAB data, indexes were obtained for the metals barium, copper and nickel, and the organic compounds chloroform, chlorpyrifos, diazinon, simazine,

terbuthylazine, tetrachloroethene and toluene. Although some compounds are repeated, a decision was made to keep both calculations for the different sources of information. Table 8.1 presents the selection of compounds and their reference values. BCFj values for organic compounds were obtained from their log Kow.

Table 8.1. Reference values (Cref) for the calculation of the impact indexes for the selected compounds (according to ACA and SGAB sources) and additional pesticides (VIECO project)

Compounds	PNEC aquatic organisms ($\mu g\ L^{-1}$)	PNEC terrestrial vertebrates ($\mu g\ kg^{-1}$)	PNEC tv/BCF ($\mu g\ L^{-1}$)	EQS-AA ($\mu g\ L^{-1}$)	EQS-MAC ($\mu g\ L^{-1}$)	Ref
2,4-Dichlorophenoxyacetic acid	500					(a)
Alachlor				0.3	0.7	[3]
Atrazine				0.6	2	[3]
Barium	58	103600	25900			(b)
Chlorfenvinphos				0.1	0.3	[3]
Chloroform	13.3	48	5.11			[29]
Chlortoluron	0.018					(c)
Chlorpyrifos				0.03	0.1	[3]
Copper	0.8					see text
Diazinon	0.003					[30]
DDE	0.0016	825	0.0602			[29]
Dimethoate	3.60					[31]
Diuron				0.2	1.8	[3]
Fluorides	400	20000	1546247			[29]
Isoproturon				0.3	1	[3]
Lindane	0.058	500	2.66	0.02	0.04	[3,29]
Malathion	0.037					[32]
MCPA	0.022					(c)
Mecoprop	124					(c)
Metolachlor	0.76					[30]
Nickel	0.6	10000	100	20		see text
Propanil	0.2					(d)
Simazine				1	4	[3]
Terbuthylazine	0.0032					[29] (c)
Tetrachloroethene	5	1160	7.49	10		[3,29]
Toluene	74	74300	2085			[29]
Zinc	1.83	11600	11.6			see text

(a) Value obtained from 3 NOECs from ECOTOX and applying an AF of 10
(b) Value obtained from 2 NOECs from ECOTOX and applying an AF of 50
(c) Values obtained from L(E)C50s from ECOTOX and applying an AF of 1000
(d) Value obtained from 2 NOECs from ECOTOX and applying an AF of 50

In the second part of the study, in the framework of a national research project called VIECO (www.proyectovieco.com), additional data was collected by analysing new samples in the same location for a list of priority and emergent pollutants, that is, pesticides and pharmaceutical products. The objective was to include new compounds not analysed routinely by public administrations or DWTP operators.

A total number of 3 sampling campaigns were performed in December 2009, March and June 2010. A total of 19 pesticides were analysed by means of an automated method based on on-line solid phase extraction (liquid chromatography–electrospray tandem mass spectrometry) (on-line SPE–LC–ESI–MS/MS) [23]. In order to choose the pesticides to be included in the study, a selection was made based on the compounds showing concentration levels above LOQ for at least 10% of the samples. Reference values were obtained as Annual Average EQS (EQS-AA) for priority pollutants and PNEC values were calculated based on toxicological data bibliographic research. Table 8.2 presents the EQS and PNEC values used in this study.

For the pharmaceutical compounds, 76 substances were analysed by off-line SPE followed by LC–ESI–MS/MS [24]. In this case, the same methodology was chosen to select the final list of compounds. Additionally, some pharmaceutical compounds had to be discarded because not enough toxicological information was available for the PNEC calculation. Table 8.2 includes PNEC values used in this work and literature references.

8.3.3. PNEC calculation

PNEC values were obtained from literature research or were calculated according to Technical Guidance on Risk Assessment [19]. This methodology is based on the idea that sensitivity of the ecosystem relies on the most sensitive species. The methodology is based on the extrapolation of toxicity tests performed in the laboratory with specific species using Assessment Factors (AF):

$$PNEC = \frac{(Test\_result)}{AF} \quad (4)$$

Table 8.2. PNEC for the studied pharmaceutical compounds and bibliographic sources for their calculation

Compounds	**PNEC** (µg/L)	**Ref.**
Acetaminophen	9.2	[33] (a)
Acetylsalicylic acid	640	[34] (i)
Atenolol	310	[35] (c)
Benzafibrate	6	[33] (a)
Betaxolol	1.5	[36] (f)
Carazolol	2.5	[36] (f)
Carbamazepine	0.5	[37] (b)
Chloroamphenicol	20	[38] (d)
Chlortetracycline	267	[36] (f)
Cimetidine	740	[39] (e)
Ciprofloxacine	0.005	[40] (c)
Clarithromycin	0.002	[41] (c)
Clenbuterol	2.0	[36] (f)
Clofibric acid	4.9	[37] (b)
Codeine	16	[36] (f)
Diazepam	4.3	[42] (c)
Diclofenac	0.1	[40] (i)
Enalapril	346	[42] (c)
Enrofloxacine	0.05	[43] (c)
Erytromicin	4.3	[33] (a)
Fenofibrate	0.78	[41] (g)
Flumequine	0.00196	[44] (c)
Fluoxetine	0.51	[33] (a)
Furosemide	100	[42] (c)
Gemfibrozil	0.9	[33] (a)
Hydrochlorothiazide	100	[42] (c)
Ibuprofen	4	[33] (a)
Indometacine	3.9	[33] (a)
Ketoprofen	32	[33] (a)
Metoprolol	7.9	[33] (a)
Metronidazole	12.5	(h)
Naproxen	15	[33] (a)
Ofloxacine	0.5	[37] (b)
Oxytetracycline	0.2	[42] (c)
Paracetamol	9.2	[42] (c)
Paroxetine	35	[45]
Phenazone	1.1	[33] (a)
Phenobarbital	50	[46] (d)
Propiphenazone	0.8	[33] (a)
Propranolol	0.01	[37] (b)
Ranitidine	63	[33] (a)
Salbutamol	240	[42] (c)
Salycilic acid	48	[33] (a)
Sulfamethazine	4	[33] (a)
Sulfamethoxazole	0.118	[40] (g)
Tetracycicline	0.09	[40] (c)
Timolol	9.0	[36] (e)
Trimethoprim	16	[33] (a)

(a) Values obtained from EC50 for fish, invertebrates (*Daphnia magna*) and algae reported at literature. Some toxicological data were estimated with ECOSAR. PNEC is being obtained from the lowest EC50 applying an AF of 1000

(b) Chronic PNEC obtained from literature

(c) Values obtained from EC50 applying an AF of 1000

(d) Value obtained from one NOEC value and applying an AF of 100

(e) Reference not available but reported at other literature sources

(f) Value obtained from EC50 assessed with ECOSAR and applying an AF of 1000

(g) Value obtained from NOECs and applying an AF of 50

(h) Value obtained from L(E)C50 from ECOTOX and applying an AF of 1000

(i) Value obtained from NOECs and applying an AF of 10

AF depends on the quantity and the type of toxicological values available. In general terms, if values are obtained from a short-term test, that is, acute exposure, e.g. EC50 (term half maximal Effective Concentration) or LC50 (term half maximal Lethal Concentration), the AF is 1000. For long-term tests to assay chronic toxicity, e.g. NOEC or LOEC (Lowest Observed Effect Concentration), the AF is between 10 and 100 depending on the number of tests and the trophic levels covered. If the number of these tests is large enough (more than 10 values relating to 8 taxonomic groups), a statistical analysis can be performed (Species Sensitivity Distribution, SSD) to find a value that protects 95% of the population [19]. In this case an AF between 1 and 5 can be applied.

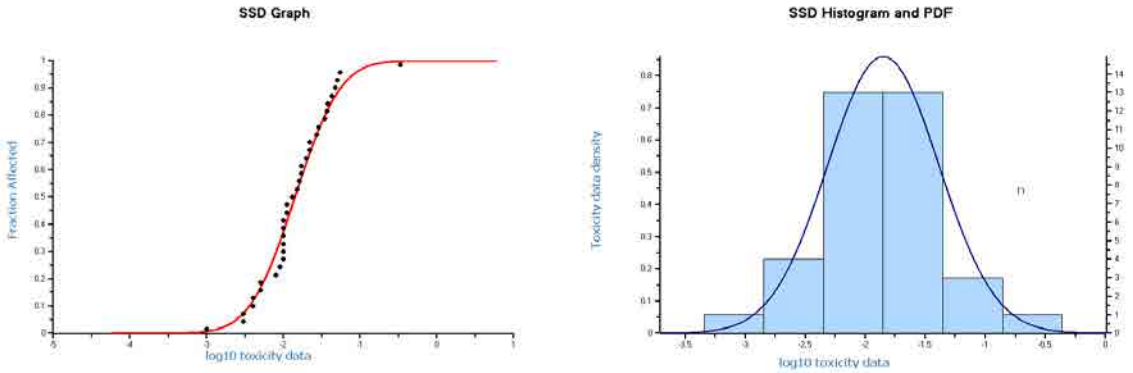
Results for PNEC calculations are shown in Table 8.1 and Table 8.2. PNEC values that were calculated following statistical analysis are described below. This analysis was performed by using version 2.0 of the software ETX from RIVM.

For copper calculation, a large amount of data was collected from different sources, belonging to algae, invertebrates and fish. After removing extreme data, the final data follows a log-normal distribution (n=43) used to estimate lower (95% confidence) HC5% values. The HC5% obtained is 2.40 μgL^{-1} for aquatic organisms with an associated confidence interval (CI) of 1.40 - 3.62 μgL^{-1}. If an AF of 3 is applied (due to the high volume of data), the resulting PNEC is 0.8 μgL^{-1}.

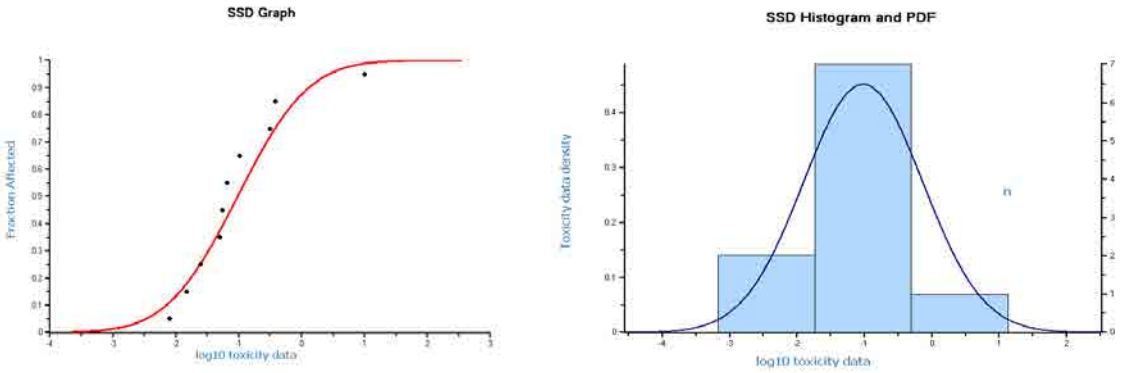
In the case of nickel, data from eleven species were obtained: three from algae, six from invertebrates and two from fish, allowing a log-normal distribution. One value was excluded for being too high, so the final volume of data was slightly reduced (n=10). The HC5% obtained is 3.02 μgL^{-1} for aquatic organisms (CI 0.26 - 1.22 μgL^{-1}). The volume of data is not as large as the case of copper so an AF of 5 was selected, resulting in a PNEC of 0.60 μgL^{-1}.

Data from 33 species belonging to the three different taxonomic groups were found for zinc PNEC calculations. One value was discarded and log-normal distribution was applied to the final volume of data (n=32). The HC5% obtained is 5.50 μgL^{-1} for aquatic organisms (CI 1.78 - 12.8 μgL^{-1}). In the case of zinc, an AF of 3 was considered appropriate. The final PNEC obtained for aquatic organisms is 1.83 μgL^{-1}. Figure 8.1 shows graphics for the sigmoidal data distribution and the histogram representing the value frequency for copper, nickel and zinc toxicity values.

(a)



(b)



(c)

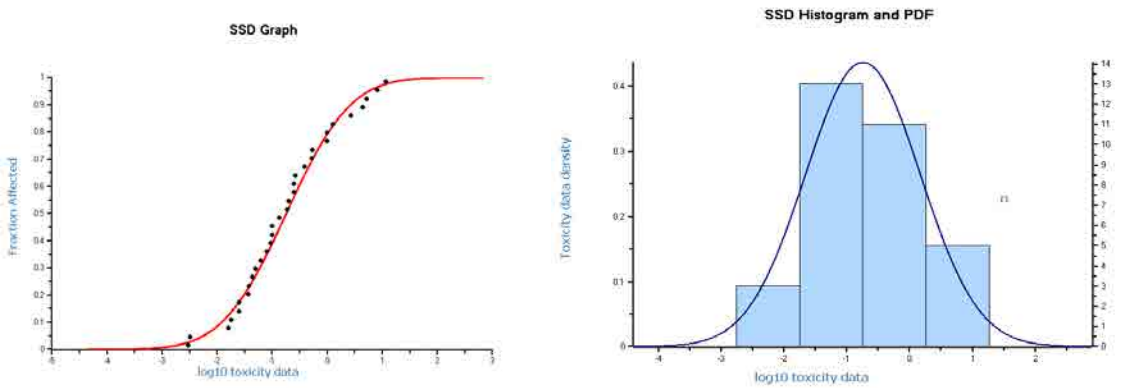


Figure 8.1 Sigmoidal log-normal cumulative data distribution and the histogram representing the value frequency for copper (a), nickel (b) and zinc (c) toxicity values. (ETX ® software)

8.4. Results and discussion

Risk and impact indexes given in the expressions (1) to (3) for ACA and SGAB data were calculated.

Figure 8.2 shows that risk indicators for aquatic organisms ($I_{ao,j}$) give significant values (index value above 1) for all metals studied, that is, barium, copper, nickel and zinc where data were recorded.

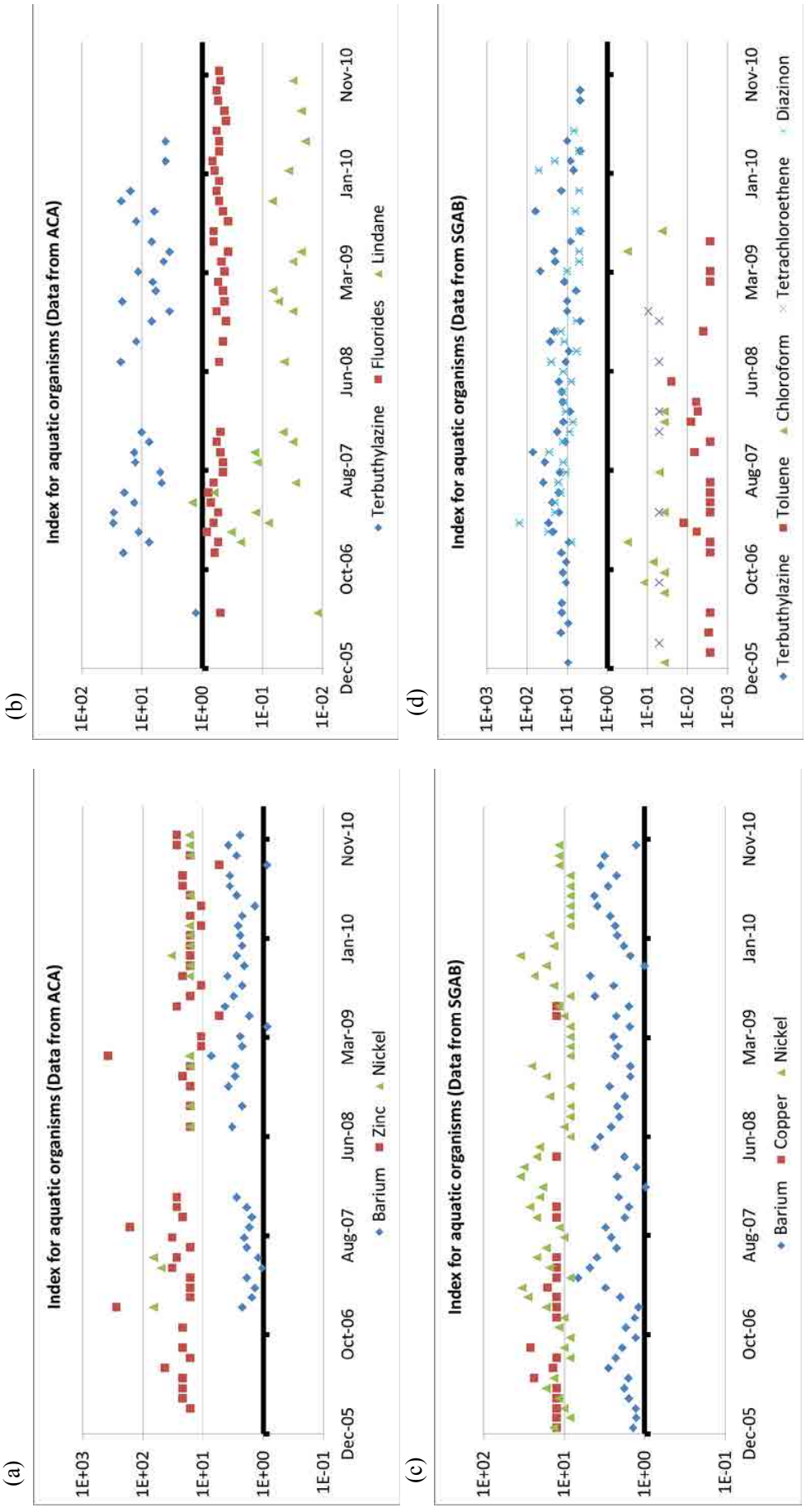


Figure 8.2. Monthly risk indicators for aquatic organisms at selected site of the Llobregat River (2006-2010) for metals (a) and organic compounds (b) in ACA data bases, and metals (c) and organic compounds (d) in SGAB data bases

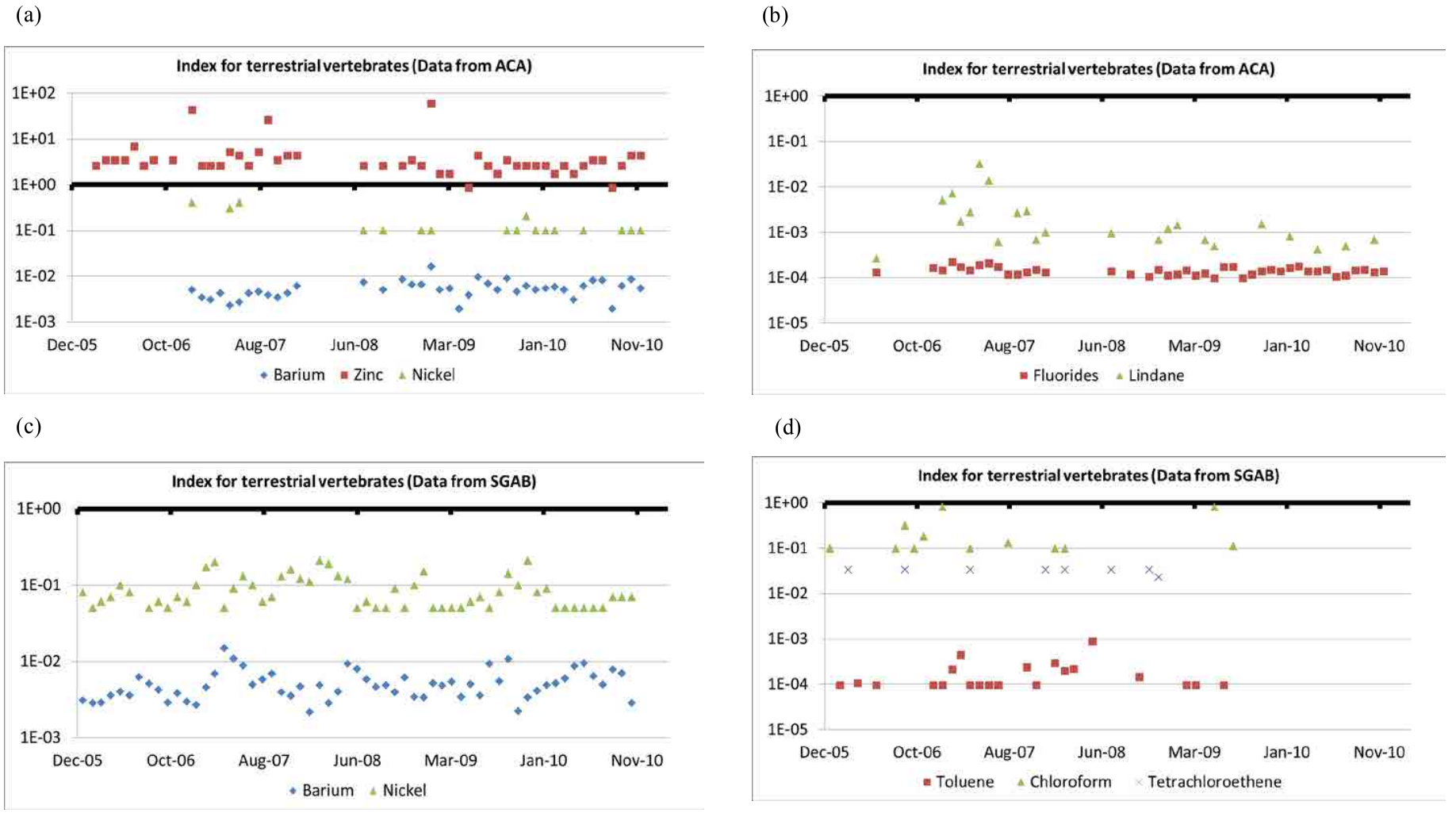


Figure 8.3. Monthly risk indicators for terrestrial vertebrates at selected site of the Llobregat River (2006-2010) for metals (a) and organic compounds (b) in ACA data bases, and metals (c) and organic compounds (d) in SGAB data bases

Only some months show values equal or below 1 (that is $1E^{+00}$) for barium. Although the toxicity of barium is lower than most of the heavy metals [25,26], the high concentrations found in Llobregat waters due to the high salinity make these indexes higher than previously thought. Zinc shows the highest indexes. Some months, the indexes reached valued between 100 and 1000 (2 and 3 orders of magnitude), posing the most serious risk for aquatic organisms. Zinc values were only obtained from ACA data bases and they refer to dissolved zinc. These higher indexes could be due to high concentration values or to very conservative PNEC values.

The uncertainty of indicators is mainly due to PNEC values derivation. As mentioned, contaminants that have little representative data have a high value of AF and use a deterministic approach, and therefore the PNEC values could be very conservative, giving indicators of one order of magnitude or more regarding a case where lots of representative data have been used. This could be the case with barium, where AF=50 was used.

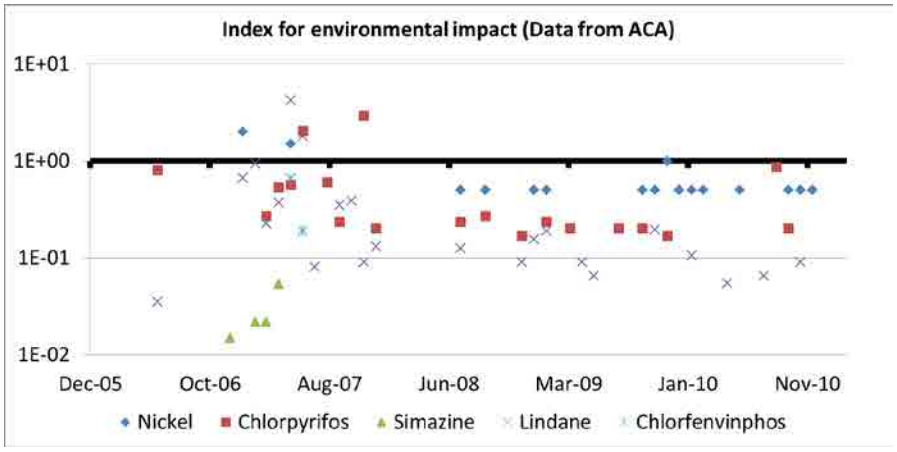
For the divalent metals copper, nickel and zinc, the derived PNEC is based on a distributed approach and therefore has less uncertainty than in the case of a deterministic approach. In the case of these metals with the range of confidence interval and range of AF (1 to 5) a variation of less than the order of magnitude in PNEC is obtained. This means that the method of derivation would not explain the high risk of indicator values. For these divalent metals, the biotic ligand model (BLM) is proposed as an alternative tool to evaluate PNEC values by considering the effect of water chemistry in the speciation and biological availability of metals in aquatic ecosystems. These models have been considered in the case of nickel, where the EQS value is much higher than derived from the PNEC in this work.

For terrestrial vertebrates, as can be seen in Figure 8.3, the only compound showing an impact according to the index calculated is zinc. Most of the monthly averages are between 1 and 10. In some cases (three specific months), the risk index is between 100 and 1000. Nickel, the only metal in the selection considered as priority pollutant, shows average values equal or a little above its EQS for some months within the studied period (Figure 8.4).

The identification of metals showing a higher risk is in accordance with other studies developing individual indexes for specific compounds. Zinc has been also prioritised in another study using toxicity indicators based on Microtox® test performed with DGT extracts [27]. Zinc and nickel were two of the inorganic compounds, along with copper, cadmium and beryllium, prioritised in another study based on SDD [28].

Concerning organic compounds, the most significant indexes for aquatic organisms refer tothe herbicide terbuthylazine, a chlorotriazine, and the nonsystemic organophosphate insecticide diazinon. In both cases, indexes are always above the threshold of 1. The values obtained are between 1 and 100 as can be seen in Figure 8.2. The other selected organic compounds show no significant index values. The indexes calculated for terrestrial vertebrates for these organic compounds show no significant impact (Figure 8.3). All these results pointed to an acceptable risk, indicating that the bioconcentration in a single chain level would not affect terrestrial vertebrates that are predators of the aquatic organisms.

(a)



(b)

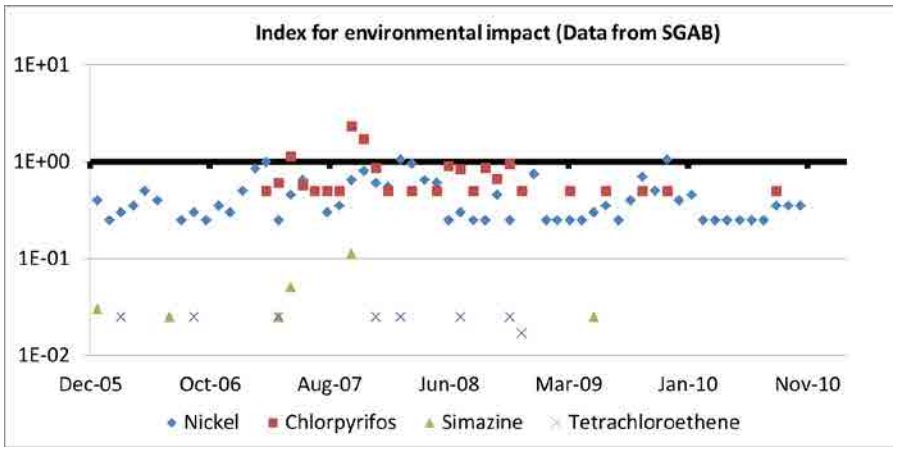


Figure 8.4. Monthly indicators for aquatic impact at selected site of the Llobregat River (2006-2010) in ACA data bases (a) and SGAB (b) data bases

Regarding aquatic impact, that is, the relation between the average concentration values and the threshold according to legislation (EQS), two compounds show indexes between 1 and 10 for some months in 2007 (Figure 8.4). These compounds are organophosphate insecticide chlorpyrifos, having applications for domestic uses, and the organochlorine insecticide γ-hexaclorociclohexane, also known as lindane. It is important to mention that these values were obtained before publication of Directive 2008/105/EC [3] which regulates their occurrence in surface waters.

Concerning VIECO data, Figure 8.5 represents obtained risk indexes for pesticides. The indexes have been calculated based on average concentrations for each compound on each campaign but whiskers show indexes based on maximum and minimum values. The only compounds showing indexes above 1 are diazinon, MCPA and terbuthylazine. The results are consistent with the ones obtained for monthly averages in the period 2006-2010 from the other sources. The indexes for the former three compounds were calculated using very low reference values compared to the EQS for the priority compounds. This is one of the reasons why non-regulated pesticides show higher risk indexes than legislated ones.

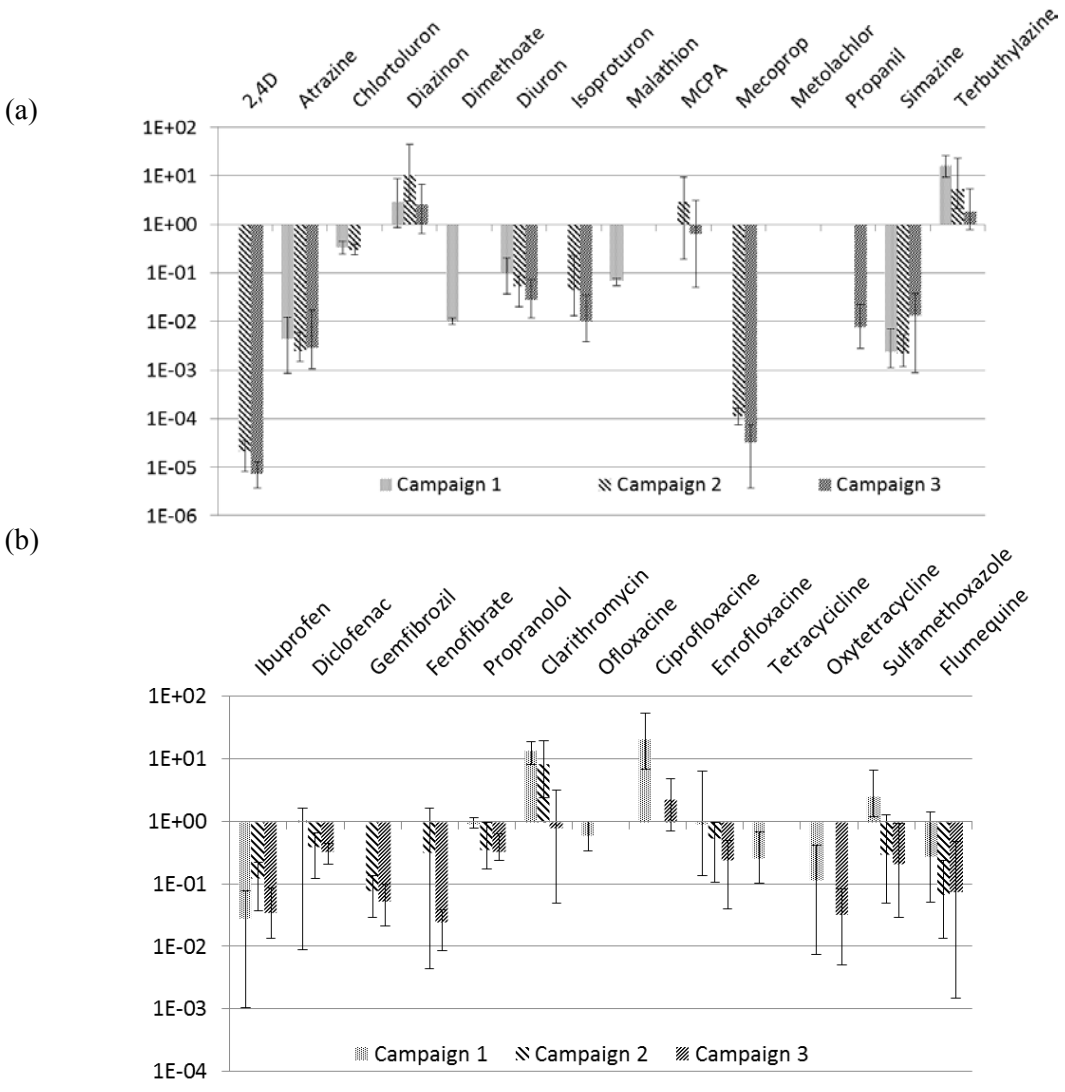


Figure 8.5. Risk indicators for pesticides (a) and pharmaceutical compounds (b) at selected site of the Llobregat River (special campaigns 2009-2010)

The risk of exposure to pesticides has been assessed in previous studies. VIECO data on pesticides concentration was published including an assessment based on Toxicity Units (TU), and toxicity values based on EC50. According to this index, diazinon, malathion and diuron were prioritised [23]. Diazinon was also highlighted in the study performed by Von der Ohe at European level [18]. Other pesticides reported to present risk were azoxystrobin, terbuthylazine, heptachlor and endosulfan. Chlorpyrifos was

the pesticide reported to show higher risk according to the study previously reported by Carafa based on SSD [28].

Figure 8.5 shows calculated indexes for the pharmaceutical compounds, showing a risk index above 0.1 in order to represent only significant values. For the antibiotics clarithromycin and ciprofloxacin the index is above 1, posing a risk to ecosystems. If indexes based on maximum concentration values are observed, the anti-inflammatory drug diclofenac, the lipid regulators gemfibrozil and fenofibrate, and the antibiotics enrofloxacin and sulfamethoxazole, present values between 1 and 10.

8.5. Conclusions

The combination of several indicators is crucial for the assessment of the river pressure based on chemical contaminants, but still there is a lack of information on reliable PNEC values. PNEC values are usually very conservative if they are not derived with the proper quantitative and qualitative data, even though they make it possible to establish the option of rejecting the negative effect of some contaminants if they do not exceed the target value.

New studies in the future will lead to more information on the toxicological effects of substances that will in turn lead to more accurate PNEC calculations and toxicological information on new compounds not currently available. An update of this study in the future could lead to different results. Additionally, if the LOQ of analytical techniques is decreased, more PEC values will be obtained.

Moreover, the potential to include new compounds in the list of priority pollutants will imply the creation of new EQS for ecological assessment indexes calculations. As can be seen in the study, EQS are less conservative than the predicted PNEC. EQS may be based on a higher number of toxicological studies than the PNEC calculated in this study. The inclusion of a substance as a priority pollutant may decrease its ecological impact according to the calculations performed.

After this initial diagnosis, more detailed studies on the effect of the potential chemicals that pose risk need to be performed as part of an ERA process in order to establish cause-effect relationships.

8.6. References

[1] 2000/60/EC, DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 October 2000 establishing a framework for Community action in the field of water policy, s. f.

[2]Agència Catalana de l'aigua, Estat de les masses d´aigua a Catalunya 2007-2009, 2010.

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9

Assessment of the Water Chemical Quality Improvement Based on Human Health Risk Indexes: Application in Drinking Water Treatment Plants incorporating Membrane Technologies

9. Assessment of the Water Chemical Quality Improvement Based on Human Health Risk Indexes: Application in Drinking Water Treatment Plants incorporating Membrane Technologies

9.1. Abstract

A methodology has been developed in order to evaluate the potential risk of drinking water for the health of consumers. The methodology used for the assessment considered systemic and carcinogenic effects caused by the oral ingestion of water based on the reference data developed by the World Health Organisation (WHO) and the Risk Assessment Information System (RAIS) for chemical contaminants. An assessment of the chemical quality improvement of produced water in the Drinking Water Treatment Plant (DWTP) after integration of membrane technologies was performed.

A series of concentration values covering up to 261 chemical paremetres over 5 years (2008-2012) of raw and treated water in the Sant Joan Despí DWTP, at the lower part of the Llobregat River basin (NE Spain), were used. After the application of the methodology, the resulting global indexes were located below the thresholds, except for carcinogenic risk in the output of DWTP, where the index was slightly above the threshold during 2008 and 2009 before the upgrade of the treatment works including membrane technologies was executed. The annual evolution of global indexes showed a reduction in the global values for all situations: HQ systemic index based on RAIS dropped from 0.64 to 0.42 for surface water and from 0.61 to 0.31 for drinking water; the R carcinogenic index based on RAIS was negligible for input water and varied between $4.2x10^{-05}$ and $7.4x10^{-06}$ for drinking water; the W systemic index based on the WHO data varied between 0.41 and 0.16 for surface water and between 0.61 and 0.31 for drinking water. A specific analysis for the indexes associated with trihalomethanes (THMs) showed the same pattern. These indexes have been presented as a tool to show the improvement of the produced water, especially after 2009 when ultrafiltration (UF) and reverse osmosis (RO) membranes were installed.

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9.2. Introduction

In developed countries, a wide implementation of water treating technologies and good management has led to a significant reduction in the risks associated with water ingestion. Good practices have led to a reduction of pollution at source and to a better removal of contaminants. In the European Union (EU), the Drinking Water Directive (98/83/EC) concerns the quality of water intended for human consumption. According to this legislation, a total of 48 microbiological, chemical and indicator paremetres must be monitored and tested regularly. Nevertheless, the list of contaminants that need to be taken into account is continuously growing as the studies to define the effects on health progress.

Water safety plans are considered by the World Health Organisation (WHO) as the most effective means of maintaining a safe supply of drinking water to the public. Hazards and risks need to be identified, and appropriate steps towards minimising these risks are then investigated (WHO, 2005). Additionally, the incidence of global driving forces, including climate change, increasing water scarcity, population growth, demographic changes and urbanisation are expected to affect the resilience of water supply and sanitation systems and services, also forcing managers to adapt their infrastructures to these driving forces (Guha-Sapir et al., 2011).

Membrane technologies have been identified as the most robust and flexible technologies used to improve water quality and taste by removing undesirable compounds and pathogens (Rahardianto et al., 2007; Reverberi and Gorenflo, 2007). Reverse osmosis (RO), nanofiltration (NF) and reverse electrodialysis (RED) are being applied worldwide to meet these needs (Birnhack and Lahav, 2007; Greenlee et al., 2009; Wang et al., 2006). The selection of a suitable membrane technology is based on technical criteria (removal of contaminants) and economic aspects (capital operation and maintenance). The implementation of new technologies in drinking water treatment plants (DWTP), such as membrane technologies, improves the quality of drinking water, as they remove toxic contaminants (Metsämuuronen et al., 2014; Radjenović et al., 2008) and reduce human health risks associated with its consumption.

However, it should be stressed that in order to ensure the minimisation of pathogens, the required treatment generates disinfection by-products (DBPs), which is one of the main drawbacks of the drinking water production. These compounds are produced by the reaction between chemical disinfectants and naturally occurring organic material (NOM), bromide, iodide, and anthropogenic pollutants present in the source water (Boorman et al., 1999; Krasner, 2009). The trihalomethanes (THMs), the most abundant DBPs, are probable human carcinogens according to the WHO (2005)based on sufficient animal evidence and inadequate human evidence of carcinogenicity. From January 1st 2009, a maximum limit of THMs of

100 μgL^{-1} was established in the EU (98/83/EC). Although values have been established for a number of DBPs, the risks associated with inadequate disinfection are far greater than the potential risks from long-term exposure to DBPs (WHO, 2014).

It is widely accepted that all stakeholders responsible for water safety should make efforts to improve risk management and risk communication to consumers, that is, the provision of information and health-based assessments on the various microbial, chemical, radiological and physical human health hazards that may be present in the water cycle. The evaluation of existing and emerging hazards in water should include a proper monitoring at source, after treatment and throughout the distribution network in order to reduce risks and an adequate approach to manage these associated risks.

Assessing exposure and the health consequences of chemicals in drinking water is challenging: exposures are typically at low concentrations, measurements in water are frequently insufficient, chemicals are present in mixtures, exposure periods are usually long, multiple exposure routes may be involved, and valid biomarkers reflecting the relevant exposure period are scarce. In addition, the magnitude of the relative risks tends to be small (Villanueva et al., 2013). Studies to assess the exposure of contaminants due to drinking water ingestion detected values of arsenic and THMs above the threshold in Turkey (Caylak, 2012) and perfluorooctane sulphonate (PFOS) in Taiwan (Chimeddulam and Wu, 2013). Industrial contamination led to high risk indexes due to metals in India (Krishna and Mohan, 2014) and Pakistan (Muhammad et al., 2011). Studies in developed countries are more focused on emerging compounds but they are limited by the availability of reference data. The risk of adverse health effects from pharmaceuticals appeared to be negligibly low in the Netherlands (Houtman et al., 2014). Schriks (2010) concluded that the majority of the compounds evaluated pose no appreciable concern individually to human health in the Rhine and Meuse Rivers. Ribera (2014) used a combination of Life Cycle Assessment (LCA) and human health risk assessment in order to select the percentage of water in DWTPs that should be nanofiltered. Results show a reduction of one order of magnitude for the carcinogenic risk index when NF produces 100% of drinking water when it is compared to direct consumption without treatment.

In this work, we have developed a methodology to determine the evolution of the chemical hazard of water. Additionally, an assessment is included on how this risk has been affected after the implementation of new treatment processes. The methodology is based on toxic effects assessment, exposure assessment and risk indexes characterisation (Durham and Swenberg, 2013). The exposure assessment in this work only considers the ingestion of drinking water containing pollutants through the oral route as the unique pathway and two typologies of effects on human health were considered: a) systemic toxicity that refers to adverse effects on any organ system following the absorption and distribution of a chemical throughout the body; and b) carcinogenic effects.

A set of water quality data recorded over five years from the DWTP monitoring programme has been used to implement the risk assessment methodology. The results obtained will be used to numerically quantify the improvement of water quality through the use of risk indexes. This study should help to develop new managing practices based not only on the occurrence, but also on the potential hazard of the chemical contaminants.

9.3. Materials and methods

9.3.1. Case study description: Llobregat River and Sant Joan Despí DWTP

In recent decades, the drinking water supply network of the Barcelona Metropolitan Area (BMA), which is 635 km^2 in size and has a population of 4.5 million inhabitants, has been primarily based on surface water resources from the Llobregat and Ter Rivers. These resources are suffering the effects of mining and industrial discharges, as well as a reduction in quantity, thereby reducing the quality of the raw water. Additionally, due to the Mediterranean climate, natural water resource availability is periodically lower than the water demand in the area (López-Roldán et al., 2013).

To improve the water quality of the Llobregat River and its tributaries, more than 30 waste water treatment plants (WWTPs) treating a mixture of urban and industrial wastewaters have been set up along the river. The main industries sited along the Llobregat River are tannery, food products, textile, pulp and paper industries, discharging a broad spectrum of organic chemicals into the river. Therefore the river receives effluents from these WWTPs and surface runoff from agricultural areas. The removal of contaminants by WWTPs is not comprehensive; consequently they can enter into the environment via sewage effluents and thus become a potential risk to the receiving bodies and in addition, to the production of drinking water (González et al., 2012; Köck-Schulmeyer et al., 2011; Valero and Arbós, 2010).

Sant Joan Despí DWTP treats water from the Llobregat River following the process flow sheet described in Figure 9.1. The plant has a maximum treatment capacity of 5.5 m^3s^{-1}, and provides almost 50% of the annual drinking water in the BMA. In 2009, an improved treatment line began its operation. The new process uses membrane technology and treats 50% of the water flow with a pre-treatment via micro-coagulation and ultrafiltration (UF) as protection for the RO step. Water is remineralised before being blended with water from the conventional treatment and sent to the post-chlorination stages. This process, the membrane treatment line according to Figure 9.1, is placed after the sand bed filtration where the flow

is split and 50% is treated with the new process; the remaining 50% will undergo ozonisation and granular activated carbon (GAC) filtration as before.

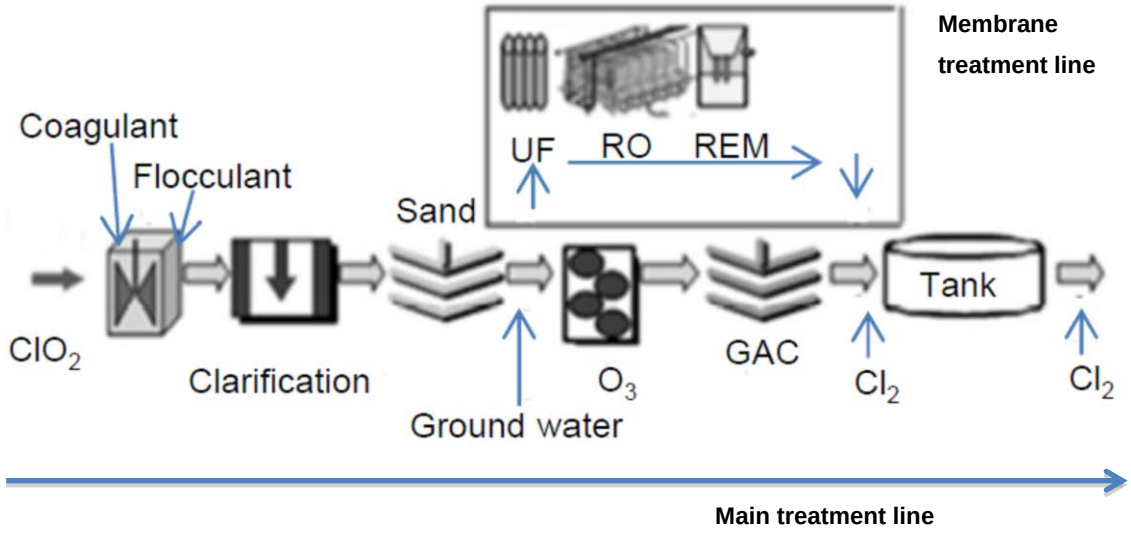

Figure 9.1. Diagram of the DWTPs involved in the study. GAC: Granular Activated Carbon; MF: Micro Filtration; REM: Remineralisation; RO: Reverse Osmosis; UF: Ultra Filtration. The box indicates the modification introduced on the treatment line including a reverse osmosis step

9.3.2. Chemical data quality collection and management

The Llobregat River has been the subject of several studies dealing with the presence of contaminants in surface water and related compartments (e.g. sediments, fish). In this work, only compounds detected in the water matrix are taken into account. Most of these studies focus on the lower and middle part of the river basin, where most of WWTPs, DWTPs and population are located, and therefore, it is the area with higher pressures. Pesticides, surfactants, oestrogens, pharmaceuticals and personal care products (PPCPs) and even illegal drugs are the main groups detected in different studies, reporting alterations in species composition or abundance, and endocrine disruption measured by alterations in enzymatic activity or specific protein production (González et al., 2012). Nevertheless, a long list of these compounds is not monitored routinely. The lack of data on their presence and their toxicity makes difficult to include them in risk assessment studies on a long-term basis. Table 9.1 provides a list of the chemical compounds routinely monitored by the Sant Joan Despí DWTP in the year 2012. The selection of the paremetres to be analysed is done based on the legislation requirements, local characteristics, occurrence according to historical data and assessment of the efficiency of treatment technologies, among other causes.

Table 9.1. Chemical paremetres routinely monitored at the Sant Joan Despí DWTP (surface water and produced drinking water) in 2012

Paremetre	Frequency inlet measurements	Frequency outlet measurements	Paremetre	Frequency inlet measurements	Frequency outlet measurements
1,1-dichloroethane	every 2 weeks	every week	Fluorides	every week	every month
1,1-dichloroethene	every year	every week	Free chlorine residual (in situ)	N/A	every hour
1,1,1-trichlorethane	every 2 weeks	every week	Gallium	every day	every day
1,1,1,2-tetrachloroethane	every year	every week	Geosmin	every 2 weeks	every 2 weeks
1,1,2-trichlorethane	every year	every week	Heptachlor	every week	every month
1,1,2,2-tetrachloroethane	every year	every week	Heptachlor epoxide	every week	every month
1,2-dibromoethane	every year	every week	Indene(1,2,3-c,d)pyrene	every week	every month
1,2-dichloroethane	every year	every week	Indium	every day	every day
1,2-dichloropropane	every year	every week	Iron	every day	every 8 hours
2-methylisoborneol	every 2 weeks	every 2 weeks	Lanthanum	every day	every day
4,4'-DDD	every week	every month	Lead	every day	every day
4,4'-DDE	every week	every month	Lindane	every week	every month
4,4'-DDT	every week	every month	Lithium	every day	every day
Acenaphthene	every week	every month	m+p-Xylene	every 2 weeks	every month
Acenaphthylene	every week	every month	Magnesium	every day	every day
Alachlor	every week	every month	Malathion	every week	every month
Aldrin	every week	every month	Manganese	every day	every day
alpha-Endosulfan	every week	every month	Mercury	every week	every day
alpha-HCH	every week	every month	Methyl parathion	every week	every month
Aluminium	every day	every 8 hours	Metolachlor	every week	every month
Ametryne	every week	every month	Molinate	every week	every month
Ammonium	every 2 hours	every 12 hours	Molybdene	every day	every day
Anthracene	every week	every month	Naphthalene	every week	every month
Antimony	every day	every day	Nickel	every 4 hours	every day
Arsenic	every day	every day	Nitrates	every week	every month
Atrazine	every week	every month	Nitrites	every week	every month
Barium	every day	every day	Non-ionic tensioactives	every 2 weeks	N/A
Benzene	every 2 weeks	every month	o-Xylene	every 2 weeks	every month
Benzo(a)anthracene	every week	every month	Palladium	every day	every day
Benzo(a)pyrene	every week	every month	Parathion	every week	every month
Benzo(b)fluorantene	every week	every month	Pendimethalin	every week	every month
Benzo(g,h,i)perylene	every week	every month	Phenanthrene	every week	every month
Benzo(k)fluorantene	every week	every month	Phenols	every 2 weeks	N/A
Beryllium	every day	every day	Phosphorus	every day	every day
beta-Endosulfan	every week	every month	Pirimicarb	every week	every month
Bismuth	every day	every day	Potassium	every day	every day
Boron	every day	every day	Prometrine	every week	every month
Bromates	every week	every day	Propanil	every week	every month
Bromides	every day	every month	Propazine	every week	every month
Bromochloroacetonitrile	N/A	every week	Pyrene	every week	every month
Bromoform	every year	every day	Rubidium	every day	every day
c-1,2-Dichloroethene	every year	every week	Selenium	every day	every day
c-1,3-Dichloropropene	every year	every week	Silicon	every day	every day
Cadmium	every day	every day	Silver	every day	every day
Calcium	every day	every 8 hours	Simazine	every week	every month
Cesium	every 2 months	every year	Sodium	every day	every day
Chlorates	N/A	every day	Strontium	every day	every day
Chlorfenvinphos	every week	every month	Sulphates	every day	every month
Chlorides	every day	every day	Sum 4 PAHs Dir. 98/83/CE	every week	every month
Chlorites	N/A	every day	Sum THMs Dir. 98/83/CE	every year	every month
Chlorodibromomethane	every year	every day	t-1,2-Dichloroethene	every year	every week
Chloroform	every year	every day	t-1,3-Dichloropropene	every year	every week
Chlorpyrifos	every week	every month	Terbuthylazine	every week	every month
Chromium	every day	every day	Terbutryn	every week	every month
Chromium (VI)	every 4 hours	every 4 hours	Tetrachloride carbon	every year	every week
Chrysene	every week	every month	Tetrachloroethene	every 2 weeks	every week
Cobalt	every day	every day	Thallium	every day	every day
Copper	every day	every day	Tin	every day	every day
Cyanides	every 8 hours	every month	Tiobencarb	every week	every month
Diazinon	every week	every month	Titanium	every day	every day
Dibenzo(a,h)anthracene	every week	every month	Toluene	every 2 weeks	every month
Dibromoacetonitrile	N/A	every week	Total Haloacetonitriles	N/A	every week
Dichlobenil	every week	every month	Total Pesticides	every week	every month
Dichloroacetonitrile	N/A	every week	Total Trihalomethanes	N/A	every day
Dichlorobromomethane	every year	every day	Trichloroacetonitrile	N/A	every week
Dieldrin	every week	every month	Trichloroethene	every 2 weeks	every week
Endrin	every week	every month	Trichloroethene + Tetrach.	every 2 weeks	every week
Ethofumesate	every week	every month	Trifluralin	every week	every month
Ethylbenzene	every 2 weeks	every month	Tungsten	every day	every day
Fenitrothion	every week	every month	Uranium	every 2 months	every year
Fluoranthene	every week	every month	Vanadium	every day	every day
Fluorene	every week	every month	Zinc	every day	every day

A series of data covering monthly averages of 261 chemical paremetres over 5 years (2008-2012) of raw and treated water in Sant Joan Despí DWTP was used. Not all paremetres were measured over the five years, as monitoring programmes were periodically adapted. Moreover, some compounds were only measured in surface water while other compounds were only measured in drinking water.

9.3.3. Fundamentals of the risk assessment methodology

Chemicals that display environmental and biological persistence, bioaccumulation, toxicity and long-range transport have been previously assessed quantitatively by national and international health agencies (Szabo and Loccisano, 2012). Among the databases that offer information on the toxicity of the compounds that can be found in water, two of the most widely used are the Risk Assessment Information System (RAIS) and the WHO guidelines (WHO, 2011).

RAIS uses the Reference Dose (RfD), expressed as an oral dose per kilogram of body weight (given in units of $mgKg^{-1}day^{-1}$), as an estimate of the lowest daily human exposure that is likely to occur without appreciable risk of deleterious, non-cancerous effects during a lifetime. WHO proposes a very similar reference value called the Tolerable Daily Intake (TDI) as an estimate of the amount of a substance in food or drinking-water, also expressed on a body-weight basis that can be ingested daily over a lifetime without any appreciable health risk (WHO, 1991). The TDI values take into account both systemic and carcinogenic effects but the risk index is calculated as systemic.

The exposure assessment of this work only considers the ingestion of drinking water containing pollutants through the oral route as the unique pathway. The oral dose for each contaminant present in water was calculated by eq 1:

$$D_i = \frac{C_w \times EF \times ED \times IR_w}{BW \times AT \times 365_{days/year}} \quad \text{(eq 1)}$$

where Di represents the dose of contaminant by water ingestion ($mg\ Kg^{-1}day^{-1}$), C_w is the annual average concentration of the contaminant in water (mgL^{-1}), EF is the exposure frequency to the contaminated media ($days\ year^{-1}$), ED is the exposure duration (year), IR_w is the rate of water intake ($L\ day^{-1}$), BW is the body weight of the receptor (Kg), and AT is the average lifetime of a person (year).

Table 9.2 shows the exposure values for the pathway of oral ingestion of water according to RAIS and WHO for the calculation of doses. For systemic risk D_i is calculated by using AT=ED.

Table 9.2. Exposure paremetres for oral ingestion of water according to RAIS and WHO

Paremetres	RAIS	WHO
EF(days year $^{-1}$)	350	365
ED* (years)	24	-
IR (L day^{-1})	2	2
BW (kg)	70	60
AT* (years)	70	-
*Systemic risk: AT=ED		

Then, three different indexes (systemic and carcinogenic for RAIS and an index for WHO) were calculated:

a) the systemic effect index according to RAIS (H_{Qi}) was calculated on the dose basis according to RAIS reference values as a ratio between the dose (D) and the dose reference level (RfD) by eq 2:

$$H_{Qi} = \frac{D_i}{R_{fDi}} \quad \text{(eq 2)}$$

where the ratio of the average daily dose to a RfD below 1 implies that adverse effects are very unlikely to occur. The guideline values were calculated separately considering the risk for individual substances, without specific consideration of additivity. Although this may result in risk underestimations, unless there is evidence to the contrary it is appropriate to assume that the toxic effects of these compounds are additive (Backhaus and Faust, 2012). Thus, a global systemic effect is obtained as contribution of the individual index values by eq 3:

$$H_Q = \sum H_{Qi} \quad \text{(eq 3)}$$

If H_Q is below 1 it implies that adverse effects are very unlikely to occur.

b) The individual carcinogenic effects were only considered in the RAIS approach and the individual carcinogenic effect index (R_i) was calculated by eq 4:

$$R_i = D_i' \times SF_i \quad \text{(eq 4)}$$

where SF is the Slope Factor (Kg day mg-1) that express a linear relationship of D_i versus the risk Ri at low doses. The cancer risk was calculated by multiplying the estimated dose or exposure level by the appropriate measure of carcinogenic potency. A guideline value of 10^{-5} means one additional cancer case per 100 000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years (Cothern et al., 1986). Following the principle of additivity of compounds, the global risk index for all compounds was calculated as an addition of individual risk indexes by eq 5:

$$R = \sum R_i \quad \text{(eq 5)}$$

c) the individual WHO (W) index was prepared by using eq 6.

$$W_i = \frac{D_i}{TDI_i} \quad \text{(eq 6)}$$

And then, the global risk index for all compounds was calculated as an addition of individual risk indexes by eq 7:

$$W = \sum W_i \quad \text{(eq 7)}$$

9.3.4. Contaminant concentrations data treatment and filtering of raw data

A tool was created and validated using the Microsoft Excel® programme for the calculation of risk indexes according to RAIS and WHO toxicity values and doses (eq 1) for the list of compounds by using eq 2-7. The tool was programmed to determine the annual average concentration of the compounds. Values representing the limit of quantitation (LOQ) for each compound were also entered so the tool was able to discriminate between measured values and values below LOQ. Blank cells were automatically recognised as non-measured paremetres in the data analysis.

The diagram in Figure 9.2 was followed in order to assess the risk related to the compounds present in water and includes filtering steps to obtain reliable risk indexes mentioned previously. When dealing with raw data concentrations in the calculation of indexes, three main issues were identified and, consequently, filtering steps were applied:

a) The lack of existence of oral toxicity data for each contaminant. The methodology was based on the risk approach, so the contaminants without toxicity values given by RAIS or WHO were excluded from

index calculations. The comparison of measured contaminants with drinking water standards set in the Directive 98/83/EC is a first step that could determine the risk when toxicity is not available.

b) Annual average concentrations were calculated using a mixture of values below the LOQ and quantified values. The election of LOQ/2 is usually applied and solves the uncertainty of a concentration that could be between zero and LOQ but, at the same time, introduces an uncertainty that has to be considered, as could lead to an overestimation of the risk (James et al., 2009). In order to have an idea of this uncertainty for the annual average values, an uncertainty index "U" was calculated by using eq 8.

$$U = 1 - \frac{Avg\ (0)}{Avg\ (LOQ/2)} \qquad \text{(eq 8)}$$

where Avg(0) is the average concentration when all the values below LOQ are considered as zero and Avg(LOQ/2) is the average concentration when all the values below LOQ are considered as LOQ/2. This U index is 1 if all the values are below LOQ (maximum uncertainty) and 0 if all the values are higher than LOQ (minimum uncertainty). The U index will be useful to evaluate the uncertainty of final global indexes.

c) Reliability of the analytical techniques for the sensitive measurement of risk indexes. It is possible that some analytical techniques are focused on the detection of contaminants just below the legal values and are not sensitive enough to calculate the contribution of the contaminants to risk assessment when present at very low concentrations. Therefore, an important role of the analytical techniques applied to risk indexes calculation would be to provide LOQ values able to quantify small amounts of risk.

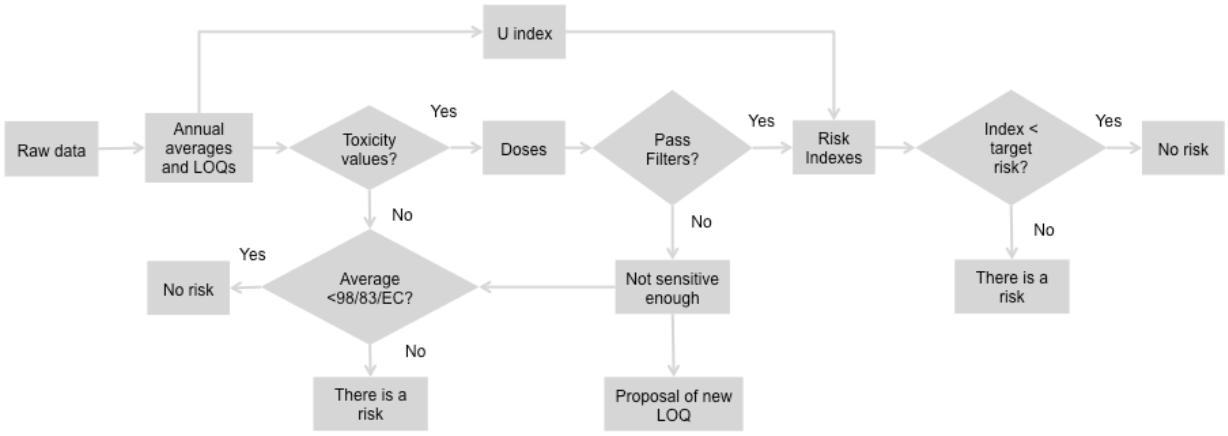

Figure 9.2. Chart flow representing the methodology for risk assessment and previous filtering steps

In order to decide which analytical techniques are sensitive enough to measure the risk properly, the calculation of the risk indexes by using LOQ levels was performed. Paremetres giving values of individual indexes, based on LOQ, below a threshold (0.02 and 0.01 for systemic risk according to RAIS and WHO, respectively and 5x10-7 for carcinogenic risk) will be included in the index. For the excluded paremetres, the risk assessment should be performed by comparing the annual average concentration with the limits recognised by legislation, as those thresholds have been also calculated on the basis of risk to human health studies.

Some extra calculations were programmed so aggregate indexes could be easily calculated taking into account some variables e.g. compounds measured at the inlet over the five years and compounds measured at the outlet over the five years. The figures showing the evolution of the final indexes have been programmed so they are automatically updated. The Excel tool has facilitated the index calculations based on a significant amount of data and can be easily adapted to new input data.

9.4. Results

9.4.1. Analysis of water quality improvement in the DWTP

Analysis of the annual evolution, from 2008 to 2012, of the average concentrations of the contaminants is included in Table 9.3 and Table 9.4. Only paremetres at the inlet and the outlet that were routinely measured over the five years have been included. As can be seen, the surface water quality of the inlet water works (Table 9.3) have improved over the years for most of the paremetres, except for arsenic, barium, cyanides, chromium, selenium, tetrachloroethene and tungsten. Other compounds such as boron, calcium, strontium, magnesium, nitrates and sulphates remain constant.

In the case of the outlet concentrations (Table 9.4), a reduction of the contaminants levels could be seen for all the compounds except for chlorates and chromium. This reduction can be explained mainly by the introduction of the RO step where at least 50% of the total waterworks capacity is treated. When the evolution of the DBPs concentrations was analysed, a reduction of 89% was achieved for total THMs. It should also be taken into account that the DWTP applied disinfection by using chlorine up to 2010 and by using chlorine dioxide from 2010, which is the main factor responsible for the formation of chlorinated DBPs. The content of chlorates has risen significantly as a DBP of the chlorine dioxide. However, the presence of bromide and iodide acted as a precursor for the formation of brominated and iodinated DBPs. Additionally, and due to the use of an ozonisation step, the formation of bromates from bromide occurred. The introduction of the membrane treatment unit improved the quality and in terms of the reduction of the high salinity (ca. 0.9 g $TDSL^{-1}$), the DBPs precursors and the DBPs themselves, so the total content of legislated THMs below 100 μgL^{-1} was easily accomplished from 2009 onwards.

When other DBPs are addressed, the increase in the chlorate content should be attributed to the substitution of the initial chlorination steps of the treatment by using chlorine dioxide instead of chlorine. Chlorate and chlorites are disinfection by products through the use of chlorine dioxide(Richardson et al., 2007). According to the WHO, a guideline value is designated as provisional because the use of chlorine dioxide as a disinfectant may result in the chlorate guideline value being exceeded, and difficulties in meeting the guideline value must never be a reason for compromising adequate disinfection. In the case of chromium (VI) the increase was due to the contribution of a groundwater pollution plume generated by electroplating industries, for more than 30 years, at industrial areas of the Llobregat Delta. The seasonal recharge of this plume onto surface water caused the detected peaks, always below the limits fixed by legislation (10 μgL^{-1}).

Table 9.3. Annual average concentrations of the compounds at the inlet and their percentage of reduction in the year 2012 compared to 2008

		2008		2009		2010		2011		2012		2008/2012
Paremetre (inlet)	Units	Avg Conc	U	Avg Conc	U	Avg Conc	U	Avg Conc	U	Avg Conc	U	Reduction*
Aluminium	$\mu g\ L^{-1}$	110.285	0.000	108.792	0.000	76.006	0.000	70.283	0.000	65.689	0.000	40%
Antimony	$\mu g\ L^{-1}$	1.386	0.030	0.864	0.289	0.500	1.000	0.750	1.000	0.750	1.000	100%
Arsenic	$\mu g\ L^{-1}$	0.838	0.298	0.816	0.358	0.927	0.225	1.024	0.488	1.851	0.068	-121%
Barium	$\mu g\ L^{-1}$	129.628	0.000	135.570	0.000	149.878	0.000	146.448	0.000	166.529	0.000	-28%
Boron	$\mu g\ L^{-1}$	198.792	0.000	154.549	0.000	175.617	0.000	196.130	0.000	211.309	0.000	-6%
Bromates	$\mu g\ L^{-1}$	16.584	0.014	6.432	0.034	2.173	0.072	2.500	1.000	2.500	1.000	100%
Bromides	$mg\ L^{-1}$	0.894	0.000	0.627	0.000	0.580	0.000	0.644	0.000	0.632	0.000	29%
Calcium	$mg\ L^{-1}$	110.442	0.000	110.509	0.000	112.958	0.000	109.647	0.000	106.272	0.000	4%
Cyanides	$\mu g\ L^{-1}$	0.542	0.846	0.500	1.000	0.500	1.000	0.815	0.460	3.905	0.320	-621%
Chlorides	$mg\ L^{-1}$	390.809	0.000	268.052	0.000	254.409	0.000	255.901	0.000	263.749	0.000	33%
Chromium	$\mu g\ L^{-1}$	2.125	0.343	1.512	0.689	1.926	0.379	1.846	0.395	2.487	0.293	-17%
Chromium (VI)	$\mu g\ L^{-1}$	4.067	0.461	2.924	0.712	3.374	0.556	2.712	0.845	2.723	0.842	33%
Diazinon	$\mu g\ L^{-1}$	0.034	0.009	0.024	0.066	0.031	0.081	0.056	0.028	0.006	0.410	82%
Strontium	$mg\ L^{-1}$	1.658	0.000	1.689	0.000	1.790	0.000	1.733	0.000	1.708	0.000	-3%
Iron	$\mu g\ L^{-1}$	61.401	0.000	55.864	0.000	57.235	0.000	47.908	0.000	50.766	0.000	17%
Phosphorus	$\mu g\ L^{-1}$	267.809	0.000	175.152	0.000	182.537	0.000	163.824	0.000	181.920	0.000	32%
Gallium	$\mu g\ L^{-1}$	1.370	0.836	1.250	1.000	1.250	1.000	1.250	1.000	1.250	1.000	100%
Geosmin	$ng\ L^{-1}$	9.492	0.077	1.250	1.000	2.500	1.000	3.727	0.610	8.250	0.253	13%
Lithium	$\mu g\ L^{-1}$	27.402	0.000	19.406	0.000	21.095	0.000	18.575	0.000	20.731	0.000	24%
Magnesium	$mg\ L^{-1}$	33.988	0.000	32.559	0.000	32.955	0.000	33.250	0.000	32.485	0.000	4%
Malathion	$\mu g\ L^{-1}$	0.006	0.573	0.004	1.000	0.004	1.000	0.004	1.000	0.004	1.000	100%
Manganese	$\mu g\ L^{-1}$	54.221	0.000	32.831	0.000	27.360	0.000	24.742	0.000	26.520	0.000	51%
Mercury	$\mu g\ L^{-1}$	0.059	0.000	0.018	0.487	0.014	0.805	0.014	0.837	0.014	0.812	76%
Molybdene	$\mu g\ L^{-1}$	2.100	0.020	1.457	0.029	1.521	0.000	1.383	0.000	1.400	0.030	33%
Nickel	$\mu g\ L^{-1}$	10.669	0.000	6.888	0.000	5.083	0.000	6.189	0.000	8.924	0.000	16%
Nitrates	$mg\ L^{-1}$	9.949	0.000	9.810	0.000	11.818	0.000	10.422	0.000	9.372	0.000	6%
Nitrites	$mg\ L^{-1}$	0.491	0.000	0.339	0.000	0.290	0.000	0.328	0.000	0.164	0.038	67%
Potassium	$mg\ L^{-1}$	37.768	0.000	26.167	0.000	24.248	0.000	26.619	0.000	29.519	0.000	22%
Rubidium	$\mu g\ L^{-1}$	30.274	0.017	13.611	0.230	21.171	0.049	9.978	0.365	12.261	0.127	59%
Selenium	$\mu g\ L^{-1}$	0.799	0.365	0.611	0.682	0.500	1.000	0.818	0.840	1.029	0.486	-29%
Silicon	$mg\ L^{-1}$	1.740	0.000	1.830	0.000	2.159	0.000	1.720	0.000	1.375	0.000	21%
Sodium	$mg\ L^{-1}$	199.810	0.000	142.642	0.000	133.269	0.000	144.095	0.000	147.260	0.000	26%
Sulphates	$mg\ L^{-1}$	170.747	0.000	167.688	0.000	176.347	0.000	170.975	0.000	164.838	0.000	3%
Thallium	$\mu g\ L^{-1}$	1.779	0.527	1.391	0.824	1.250	1.000	1.603	0.585	1.486	0.701	16%
Non-ionic tensioactives	$mg\ L^{-1}$	0.090	0.000	0.068	0.000	0.092	0.000	0.081	0.000	0.083	0.000	8%
Terbuthylazine	$\mu g\ L^{-1}$	0.042	0.000	0.072	0.000	0.012	0.161	0.020	0.108	0.018	0.136	56%
Terbutryn	$\mu g\ L^{-1}$	0.007	0.796	0.005	0.665	0.004	1.000	0.004	0.795	0.004	1.000	100%
Tetrachloroethene	$\mu g\ L^{-1}$	0.138	0.264	0.260	0.022	0.125	1.000	0.145	0.788	0.156	0.735	-13%
Titanium	$\mu g\ L^{-1}$	1.938	0.000	1.837	0.023	1.232	0.135	1.745	0.072	1.608	0.078	17%
Total Pesticides	$\mu g\ L^{-1}$	0.150	0.764	0.125	1.000	0.141	0.815	0.141	0.007	0.020	0.063	87%
Vanadium	$\mu g\ L^{-1}$	2.388	0.000	1.492	0.056	1.295	0.064	1.137	0.073	1.002	0.166	58%
Tungsten	$\mu g\ L^{-1}$	2.241	0.232	1.495	0.697	1.368	0.838	1.837	0.397	3.332	0.031	-49%

*Percentage of reduction of the concentration of the paremetre comparing 2008 and 2012. 100% of reduction means that the concentration in 2012 has fallen below LOQ

Table 9.4. Annual average concentrations of the compounds at the outlet and their percentage of reduction in the year 2012 compared to 2008

Paremetre (outlet)	Units	2008 Avg Conc	U	2009 Avg Conc	U	2010 Avg Conc	U	2011 Avg Conc	U	2012 Avg Conc	U	2008/2012 Reduction*
1,1,1-trichlorethane	µg L^{-1}	0.200	0.042	0.025	1.000	0.025	1.000	0.125	1.000	0.125	1.000	100%
1,1,2-trichlorethane	µg L^{-1}	3.857	0.001	0.397	0.026	1.117	0.000	1.939	0.000	2.265	0.009	41%
1,2-dichloroethane	µg L^{-1}	0.281	0.111	0.119	0.480	0.083	0.630	0.225	1.000	0.225	1.000	100%
Aluminium	µg L^{-1}	44.761	0.000	86.387	0.000	57.931	0.000	53.135	0.000	34.908	0.000	22%
Antimony	µg L^{-1}	0.564	0.812	0.551	0.831	0.500	1.000	0.750	1.000	0.750	1.000	100%
Silver	µg L^{-1}	0.669	0.560	0.500	1.000	0.500	1.000	0.750	1.000	0.750	1.000	100%
Barium	µg L^{-1}	54.383	0.000	52.663	0.000	36.219	0.000	31.276	0.000	31.261	0.000	43%
Boron	µg L^{-1}	195.303	0.000	129.547	0.000	154.589	0.000	137.830	0.000	147.601	0.000	24%
Bromates	µg L^{-1}	5.745	0.011	7.893	0.000	7.867	0.008	3.636	0.458	4.161	0.350	28%
Bromoform	µg L^{-1}	39.647	0.000	35.545	0.000	19.724	0.000	20.063	0.000	16.126	0.000	59%
Bromides	mg L^{-1}	0.136	0.123	0.216	0.029	0.165	0.076	0.097	0.107	0.074	0.198	46%
Calcium	mg L^{-1}	134.535	0.000	108.935	0.000	97.789	0.000	92.806	0.000	90.362	0.000	33%
Free chlorine residual (in situ)	mg L^{-1}	1.006	0.000	1.007	0.000	0.837	0.000	0.828	0.000	0.858	0.000	15%
Total chlorine residual (in situ)	mg L^{-1}	1.192	0.000	1.183	0.000	0.967	0.000	0.931	0.000	0.980	0.000	18%
Chlorates	µg L^{-1}	85.201	0.039	954.511	0.001	1012.222	0.000	857.978	0.000	822.752	0.000	-866%
Chlorites	µg L^{-1}	13.000	0.353	13.339	0.187	17.021	0.171	14.978	0.111	9.026	0.231	31%
Chlorodibromomethane	µg L^{-1}	28.842	0.000	19.148	0.000	6.204	0.000	4.891	0.000	4.277	0.000	85%
Chloroform	µg L^{-1}	9.373	0.000	6.023	0.000	0.483	0.000	0.745	0.000	0.619	0.000	93%
Chlorides	mg L^{-1}	414.610	0.000	280.842	0.000	186.797	0.000	179.581	0.000	183.703	0.000	56%
Cobalt	µg L^{-1}	0.625	0.667	0.500	1.000	0.500	1.000	0.500	1.000	0.500	1.000	100%
Chromium	µg L^{-1}	2.171	0.384	1.956	0.479	2.631	0.277	2.239	0.326	3.921	0.027	-81%
Chromium (VI)	µg L^{-1}	2.500	1.000	2.922	0.713	3.240	0.579	3.623	0.403	3.073	0.678	-23%
Dibromoacetonitrile	µg L^{-1}	0.281	0.119	0.050	1.000	0.102	0.287	0.050	1.000	0.089	0.469	68%
Dichlorobromomethane	µg L^{-1}	18.654	0.000	10.881	0.000	0.797	0.000	0.633	0.000	0.634	0.000	97%
Strontium	mg L^{-1}	1.852	0.000	1.626	0.000	1.226	0.000	1.098	0.000	1.103	0.000	40%
Ethylbenzene	µg L^{-1}	0.038	0.000	0.045	0.000	0.050	1.000	0.125	1.000	0.250	1.000	100%
Iron	µg L^{-1}	9.205	0.000	8.831	0.000	8.999	0.000	8.389	0.000	8.751	0.014	5%
Fluorides	mg L^{-1}	0.151	0.000	0.114	0.055	0.119	0.035	0.127	0.016	0.106	0.059	29%
Phosphorus	µg L^{-1}	24.928	0.017	13.242	0.063	10.617	0.196	11.174	0.149	13.343	0.094	46%
Lithium	µg L^{-1}	28.483	0.000	17.996	0.000	13.654	0.000	11.287	0.000	12.522	0.000	56%
m+p-Xylene	µg L^{-1}	0.071	0.000	0.052	0.000	0.050	1.000	0.250	1.000	0.500	1.000	100%
Magnesium	mg L^{-1}	43.063	0.000	31.344	0.000	24.860	0.000	22.281	0.000	23.147	0.000	46%
Manganese	µg L^{-1}	1.920	0.000	1.176	0.142	1.061	0.118	0.901	0.185	0.995	0.168	48%
Mercury	µg L^{-1}	0.046	0.045	0.014	0.794	0.013	1.000	0.013	1.000	0.013	1.000	100%
Molybdene	µg L^{-1}	1.104	0.189	0.975	0.214	0.500	1.000	0.846	0.246	0.636	0.589	42%
Nickel	µg L^{-1}	5.108	0.000	3.263	0.000	2.527	0.000	2.747	0.000	3.763	0.000	26%
Nitrates	mg L^{-1}	10.755	0.001	10.313	0.000	9.138	0.000	7.700	0.000	7.458	0.000	31%
Potassium	mg L^{-1}	34.802	0.000	23.162	0.000	16.988	0.000	17.182	0.000	19.175	0.000	45%
Rubidium	µg L^{-1}	18.471	0.056	9.556	0.436	8.933	0.525	8.302	0.565	7.398	0.704	60%
Selenium	µg L^{-1}	0.569	0.805	0.500	1.000	0.500	1.000	0.876	0.713	0.750	1.000	100%
Silicon	mg L^{-1}	2.507	0.000	1.643	0.000	1.463	0.000	1.169	0.000	1.249	0.000	50%
Sodium	mg L^{-1}	203.257	0.000	129.339	0.000	98.539	0.000	99.359	0.000	106.460	0.000	48%
Sulphates	mg L^{-1}	197.292	0.000	158.333	0.000	127.833	0.000	110.333	0.000	109.917	0.000	44%
Thallium	µg L^{-1}	1.446	0.793	1.406	0.815	1.250	1.000	1.474	0.707	1.474	0.706	-2%
Terbuthylazine	µg L^{-1}	0.005	0.708	0.004	1.000	0.004	1.000	0.004	1.000	0.004	1.000	100%
Tetrachloroethene	µg L^{-1}	0.395	0.047	0.084	0.822	0.119	0.472	0.125	1.000	0.213	0.342	46%
Toluene	µg L^{-1}	0.063	0.000	0.093	0.000	0.080	0.573	0.250	1.000	0.250	1.000	100%
Total Haloacetonitriles	µg L^{-1}	0.414	0.242	0.150	1.000	0.243	0.360	0.150	1.000	0.188	0.733	55%
Total Trihalomethanes	µg L^{-1}	96.449	0.000	46.296	0.000	13.267	0.000	12.766	0.000	11.044	0.000	89%
Trichloroethene	µg L^{-1}	0.562	0.000	0.173	0.000	0.356	0.000	0.249	0.293	0.163	0.638	71%
Trichloroethene + Tetrachloroethene	µg L^{-1}	0.953	0.000	0.305	0.000	0.301	0.249	0.360	0.521	0.317	0.658	67%
Vanadium	µg L^{-1}	1.067	0.234	0.835	0.349	0.558	0.821	0.586	0.711	0.500	1.000	100%

*Percentage of reduction of the concentration of the paremetre comparing 2008 and 2012. 100% of reduction means that the concentration in 2012 has fallen below LOQ

9.4.2. Risk indexes comparison of raw and treated water

Global indexes for systemic risk according to RAIS and WHO reference values and carcinogenic risk based on RAIS reference data are shown in Table 9.5. The global risks indexes have been calculated through the addition of the individual indexes. For the calculation of the global indexes, only the compounds that were measured over the five years at the surface water (inlet) on one side, and the compounds measured for the five years at the treated water (outlet) on the other side have been includedin order to obtain comparable global indexes.

Table 9.5. Global risk indexes calculation for systemic risk according to RAIS reference values (H_Q) and WHO values (W) and carcinogenic risk (R)

	Inlet			Outlet		
	H_Q	R	W	H_Q	R	W
2008	0.64	$4.62x10^{-07}$	0.41	0.61	$4.21x10^{-05}$	0.32
2009	0.50	$4.51x10^{-07}$	0.31	0.50	$2.66x10^{-05}$	0.25
2010	0.49	$4.68x10^{-07}$	0.30	0.41	$8.18x10^{-06}$	0.19
2011	0.47	$5.11x10^{-07}$	0.32	0.33	$8.06x10^{-06}$	0.17
2012	0.42	$5.24x10^{-07}$	0.16	0.31	$7.40x10^{-06}$	0.17

A list of compounds is not included in the global index due to the filtering steps performed, because of the unavailability of reference data or the low sensitivity of the analytical technique. If they are excluded, the annual average concentrations are compared to the thresholds established in Directive 98/83/EC. Table 9.6, Table 9.7 and Table 9.8 show the concentrations of these paremetres for the outlet water in the calculation of H_Q, R and W. Only free chlorine (2008-2009), chlorides (2008-2009) and sodium (2008) show levels above Directive reference values.

Figure 9.3 shows the annual evolution of the global indexes. Thresholds for the three types of indexes have also been included (H_Q<1; R<10^{-5}; W<1). It should be highlighted that, although the thresholds were designed for individual paremetres, they were applied in this methodology to the global risk values. The annual evolution of indexes shows a reduction in the global risk for all situations. The biggest reduction can be seen after the first year, 2008, when a severe drought took place. The low average river flow in 2008 (8.12 m^3s^{-1} compared to 12.83 m^3s^{-1}in 2009) may be associated to higher average concentrations of pollutants and, therefore, an increase of the risk indexes.

Table 9.6. Annual averages of the compounds excluded from the H_Q calculation when there are reference values from Directive 98/83/CE

Parametre (excluded from H_Q)	Unit	2008	2009	2010	2011	2012
Antimony	µg L^{-1}	0.564	0.551	0.500	0.750	0.750
Arsenic	µg L^{-1}	0.558	0.500	0.500	0.750	0.907
Bromates	µg L^{-1}	-	-	-	3.636	4.161
Free chlorine residual (in situ)	mg L^{-1}	1.006*	1.007*	0.837	0.828	0.858
Chlorides	mg L^{-1}	414.610*	280.842*	186.797	179.581	183.703
Chromium	µg L^{-1}	2.171	1.956	2.631	2.239	3.921
Fluorides	mg L^{-1}	0.151	0.114	0.119	0.127	0.106
Lindane	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Pirimicarb	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Sodium	mg L^{-1}	203.257*	129.339	98.539	99.359	106.460
Sulphates	mg L^{-1}	197.292	158.333	127.833	110.333	109.917
Terbuthylazine	µg L^{-1}	0.005	0.004	0.004	0.004	0.004
Trichloroethene + Tetrachloroethene	µg L^{-1}	0.953	0.305	0.301	0.360	0.317

*Average concentrations above the thresholds established in Directive 98/83/EC: free chlorine (1 mg/L), chlorides (250 mg/L) and sodium (200 mg/L)

Table 9.7. Annual averages of the compounds excluded from the R calculation when there are reference values from Directive 98/83/CE

Parametre (excluded from R)	Unit	2008	2009	2010	2011	2012
Aldrin	µg L^{-1}	0.004	0004	0.004	0.004	0.004
alpha-Endosulfan	µg L^{-1}	0.006	0.004	0.004	0.004	0.005
Aluminium	µg L^{-1}	44.761	86.387	57.931	53.135	34.908
Ammonium	mg L^{-1}	0.038	0.038	0.041	0.038	0.038
Antimony	µg L^{-1}	0.564	0.551	0.500	0.750	0.750
Arsenic	µg L^{-1}	0.558	0.500	0.500	0.750	0.907
beta-Endosulfan	µg L^{-1}	0.004	0.004	0.004	0.004	0.006
Boron	µg L^{-1}	195.303	129.547	154.589	137.830	147.601
Bromates	µg L^{-1}	5.745	7.893	7.867	3.636	4.161
Free chlorine residual (in situ)	mg L^{-1}	1.006*	1.007*	0.837	0.828	0.858
Chlorfenvinphos	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Chlorides	mg L^{-1}	414.610*	280.842*	186.797	179.581	183.703
Chromium	µg L^{-1}	2.171	1.956	2.631	2.239	3.921
Dieldrin	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Iron	µg L^{-1}	9.205	8.831	8.999	8.389	8.751
Fluorides	mg L^{-1}	0.151	0.114	0.119	0.127	0.106
Heptachlor	µg L^{-1}	0.006	-	-	-	-
Heptachlor epoxide	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Lindane	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Malathion	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Manganese	µg L^{-1}	1.920	1.176	1.061	0.901	0.995
Mercury	µg L^{-1}	0.046	0.014	0.013	0.013	0.013
Metolachlor	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Molinate	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Nickel	µg L^{-1}	5.108	3.263	2.527	2.747	3.763
Nitrates	mg L^{-1}	10.755	10.313	9.138	7.700	7.458
Nitrites	mg L^{-1}	0.010	0.010	0.010	0.010	0.010
Pendimethalin	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Pirimicarb	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Propazine	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Selenium	µg L^{-1}	0.569	0.500	0.500	0.876	0.750
Sodium	mg L^{-1}	203.257*	129.339	98.539	99.359	106.460
Sulphates	mg L^{-1}	197.292	158.333	127.833	110.333	109.917
Terbuthylazine	µg L^{-1}	0.005	0.004	0.004	0.004	0.004
Terbutryn	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Tiobencarb	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Trichloroethene + Tetrachloroethene	µg L^{-1}	0.953	0.305	0.301	0.360	0.317

*Average concentrations above the thresholds established in Directive 98/83/EC: free chlorine (1 mg/L), chlorides (250 mg/L) and sodium (200 mg/L)

Table 9.8. Annual averages of the compounds excluded from the W calculation when there are reference values from Directive 98/83/CE

Parametre (excluded from W)	Unit	2008	2009	2010	2011	2012
1,2-dichloroethane	µg L^{-1}	0.281	0.119	0.083	0.225	0.225
Alachlor	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
alpha-Endosulfan	µg L^{-1}	0.006	0.004	0.004	0.004	0.005
Aluminium	µg L^{-1}	44.761	86.387	57.931	53.135	34.908
Ammonium	mg L^{-1}	0.038	0.038	0.041	0.038	0.038
Arsenic	µg L^{-1}	0.558	0.500	0.500	0.750	0.907
Atrazine	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Benzene	µg L^{-1}	0.023	0.035	0.050	0.125	0.125
beta-Endosulfan	µg L^{-1}	0.004	0.004	0.004	0.004	0.006
Bromates	µg L^{-1}	5.745	7.893	7.867	3.636	4.161
Free chlorine residual (in situ)	mg L^{-1}	1.006*	1.007*	0.837	0.828	0.858
Chlorfenvinphos	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Chlorides	mg L^{-1}	414.610*	280.842*	186.797	179.581	183.703
Chromium	µg L^{-1}	2.171	1.956	2.631	2.239	3.921
Iron	µg L^{-1}	9.205	8.831	8.999	8.389	8.751
Fluorides	mg L^{-1}	0.151	0.114	0.119	0.127	0.106
Heptachlor	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Heptachlor epoxide	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Malathion	µg L^{-1}	0.004	0.004	0.004	0.004	0.004
Manganese	µg L^{-1}	1.920	1.176	1.061	0.901	0.995
Pirimicarb	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Propazine	µg L^{-1}	0.006	0.006	0.006	0.006	0.006
Selenium	µg L^{-1}	0.569	0.500	0.500	0.876	0.750
Sodium	mg L^{-1}	203.257*	129.339	98.539	99.359	106.460
Sulphates	mg L^{-1}	197.292	158.333	127.833	110.333	109.917
Terbutryn	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Tiobencarb	µg L^{-1}	0.006	0.004	0.004	0.004	0.004
Trichloroethene + Tetrachloroethene	µg L^{-1}	0.953	0.305	0.301	0.360	0.317

*Average concentrations above the thresholds established in Directive 98/83/EC: free chlorine (1 mg/L), chlorides (250 mg/L) and sodium (200 mg/L)

From the methodology developed it is also possible to identify the main contaminants contributing to risk. Lists for the top 10 compounds contributing to every risk index for the year 2012 are shown in Table 9.9. U indexes show the uncertainty related to the calculation of the individual risks. The closer U is to 1, the higher the uncertainty of the value of the annual concentration used for the risk calculation. The compounds posing a major risk show U close to 0, except for the carcinogenic risk at the inlet, which is based on compounds not found (U=1), although the global index shows an acceptable risk.

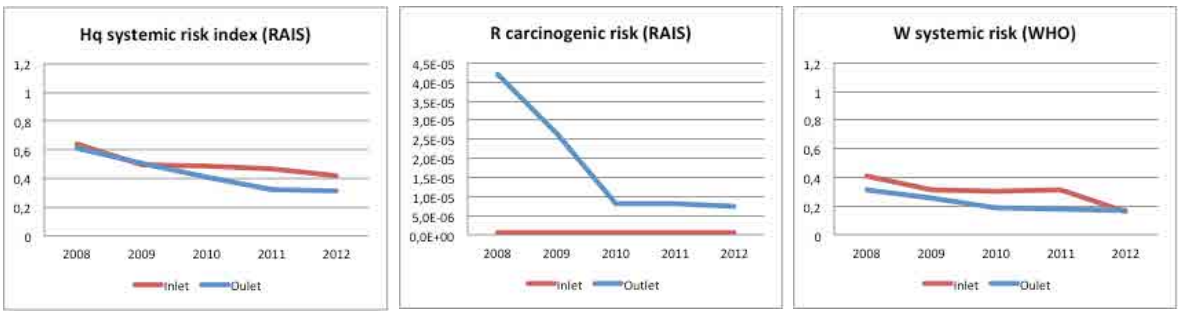

Figure 9.3. Annual evolution of the global indexes for systemic and carcinogenic risk assessment according to RAIS and WHO reference data

For systemic risk according to RAIS reference values, H_Q, the compounds posing a major risk at the inlet are nitrates and nitrites. Nitrates are found at high concentrations and the main risk is linked to their potential of becoming nitrites. Some other compounds such as bromates, strontium, boron, barium and nickel also contribute considerably to the global risk. At the outlet, boron and the halogenated chlorodibromomethane, bromoform, trichloroethene and 1,1,2-trichlorethane appear at the highest position in the list along with the above compounds. Some other compounds such as fluorides, free chlorine and metals such as thallium, lithium and chromium (VI) and non-metals such as arsenic and antimony do not contribute to the global index calculation as the techniques for performing the analysis were regarded as not sensitive enough. Therefore, this indicates that LOQs need to be improved so they can be included in the global risk assessment.

In the situation according to the systemic risk index based on WHO values, W, is not so different to the index based on RAIS values, H_Q, regarding the compounds showing the highest contribution to the global index. In this case, the filter has only excluded the pesticide atrazine for the inlet plus free chlorine, chlorates and chlorites in drinking water.Chlorates deserve special attention due to the significant concentration values present in drinking water. WHO guidelines present provisional guidelines due to the effects shown in animals (Richardson et al., 2007), although further studies should be performed in order to assess the effects in humans.

Table 9.9. Lists of the compounds making a higher contribution to every risk index and their associated uncertainty in the year 2012

Risk indexes at the inlet of the DWTP

Parametre	Hqi	U	Parametre	Ri	U	Parametre	Wi	U
Nitrates	0.160	0.00	Heptachlor	$1.59x10^{-07}$	1.00	Nitrates	0.084	0.00
Strontium	0.078	0.00	Benzo(a)pyrene	$8.57x10^{-08}$	1.00	Boron	0.041	0.00
Nitrites	0.045	0.04	Dibenzo(a,h)anthracene	$8.57x10^{-08}$	1.00	Nickel	0.025	0.00
Boron	0.029	0.00	Benzene	$6.46x10^{-08}$	1.00	Antimony	0.004	1.00
Barium	0.023	0.00	Trichloroethene	$5.40x10^{-08}$	1.00	Trichloroethene	0.003	1.00
Nickel	0.012	0.00	Ethylbenzene	$2.58x10^{-08}$	1.00	Aldrin	0.001	1.00
Heptachlor epoxide	0.008	1.00	Atrazine	$1.35x10^{-08}$	1.00	Dieldrin	0.001	1.00
Molybdene	0.008	0.03	Benzo(a)anthracene	$8.57x10^{-09}$	1.00	Simazine	0.000	1.00
Beryllium	0.007	1.00	Benzo(b)fluorantene	$8.57x10^{-09}$	1.00	Tetrachloroethene	0.000	0.74
Trichloroethene	0.007	1.00	Indene(1,2,3-c,d)pyrene	$8.57x10^{-09}$	1.00	Terbuthylazine	0.000	0.14

Risk indexes at the outlet of the DWTP

Parametre	Hqi	U	Parametre	Ri	U	Parametre	Wi	U
Nitrates	0.128	0.00	Chlorodibromomethane	$3.37x10^{-06}$	0.00	Nitrates	0.067	0.00
Strontium	0.050	0.00	1,1,2-trichlorethane	$1.21x10^{-06}$	0.01	Bromoform	0.030	0.00
Bromoform	0.022	0.00	Bromoform	$1.20x10^{-06}$	0.00	Boron	0.029	0.00
Boron	0.020	0.00	Dichlorobromomethane	$3.70x10^{-07}$	0.00	Nickel	0.010	0.00
1,1,2-trichlorethane	0.016	0.01	1,1,2,2-tetrachloroethane	$2.35x10^{-07}$	1.00	Chlorodibromomethane	0.007	0.00
Trichloroethene	0.009	0.64	1,2-dichloroethane	$1.92x10^{-07}$	1.00	Nitrites	0.005	1.00
Heptachlor epoxide	0.008	1.00	Chloroform	$1.80x10^{-07}$	0.00	Antimony	0.004	1.00
Beryllium	0.007	1.00	1,2-dichloropropane	$1.69x10^{-07}$	1.00	Trichloroethene	0.004	0.64
Chlorodibromomethane	0.006	0.00	Heptachlor	$1.59x10^{-07}$	1.00	Tetrachloride carbon	0.003	1.00
Nickel	0.005	0.00	Tetrachloride carbon	$8.22x10^{-08}$	1.00	Chloroform	0.001	0.00

For the carcinogenic risk, R, no compounds have a significant risk at the inlet, and values are two order of magnitude below the threshold. The risk is higher at the outlet as DBPs can only be found in the treated water. Compounds such as bromates, chromium and arsenic were discounted as contributing to the risk to a large extent due to their high LOQs but were below the values established in Directive 98/83/CE.

At the outlet, legislated THMs and 1,1,2-Trichloroethane make the maximum contribution to the general added risk. A reduction in the risk is especially significant after 2009. This improvement is associated with the implementation of the RO treatment step, where a reduction in the concentrations of the DBPs precursors (bromide, iodide and dissolved organic matter) is expected, along with a reduction in the DBP concentration formed in the chlorination step before coagulation (see Figure 9.1). This reduction is shown in Figure 9.4 where the evolution of the levels of THMs is shown.

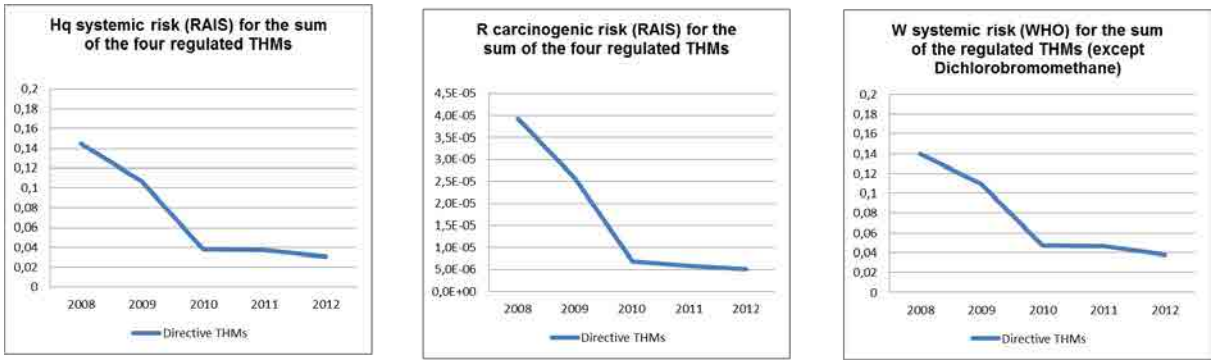

Figure 9.4. Annual evolution of risk indexes for systemic and carcinogenic risk assessment for the four regulated THMs (bromoform, chlorodibromomethane, chloroform, dichlorobromomethane) at the outlet

9.4.3. Contribution of disinfection by-products on risk indexes

A special analysis was made of disinfection by-products in drinking water. The four THMs included in the legislation (bromoform, bromodichloromethane, dibromochloromethane, chloroform) are some of the top-ranking compounds in contributing to the global carcinogenic risk. Figure 9.4 shows the decrease of risk over time due mainly to a reduction of the concentration of THMs. It should be stated that for the four regulated THMs, U is equal to 0, showing no uncertainty in the risk calculations as the compounds are always quantified above their LOQs.

This reduction can be explained by two main factors: the upgrade of the treatment line through the inclusion of the RO desalination treatment in 2009 and the replacement of chlorine by chlorine dioxide with a weaker oxidation potential, and therefore, with a lower capacity for the formation of DBPs. The introduction of a desalination step treating 50% of the in-let flow rate reduced the concentration of the

DBPs precursors, both inorganic species such as bromide and iodide, and organic species, mainly dissolved organic matter (natural and non-natural). The reduction of the DBP concentrations by up to four times could only be explained by the combination of both changes on the treatment line.

Not all DBPs have decreased as it has been highlighted in section 9.4.1. Chlorates have increased due a change in the disinfection process where the use of chlorine was substituted by chlorine dioxide. Chlorates are on of the main DBP generated because of the use of chlorine dioxide. Due to the fact that chlorates have been included in the group of compounds whose risk is not included in the aggregated value, the contribution cannot be seen in the global index. Due to the fact that effects in animals have been proved (Richardson et al., 2007), it is important to monitor this compound and follow its evolution. An effort to optimize the dosing of chlorine dioxide in the plant to reduce the levels of chlorate started in 2014, with the objective of achieving values below the guideline value 0.7 mg/L. Additionally, a new initiative to improve the analytical methodologies of chlorate analysis reducing the LOQ is under development.

9.4.4. Risk indexes methodology advantages and constraints.

The main advantage of these calculations is related to the fact that they are based on three accepted approaches to the assessment of health risks, differentiating between systemic and carcinogenic risk. These indexes consider all measured paremetres even if the monthly average concentrations are below the LOQ. The obtained indexes can be recalculated as long as new substances are analysed and WHO or RAIS recognises new toxicity values.

But as these global indexes include individual values of specific pollutants, it is noticeable that the final risk values increase as new paremetres are measured, even if the results of the analytics are below the quantification limit. In order to cope with this problem, it is important to establish filters so substances presenting high-risk values when concentrations are below quantification limits do not overestimate global health risks.

A series of data shows some limitations when the methodology is applied. Monthly averages are calculated on the base of the different frequency of measurements depending on the specific paremetre, so the number of analysis values and the time when they were performed can have an influence on the results. Additionally, the same list of paremetres has not been recorded over the 5 years. In order to be able to perform an annual comparison, global indexes only include the paremetres that have been measured over the five years covered in the study.

Analytical techniques have their own constraints, as no concentration values can be reported under LOQ. This LOQ is not only dependent on the technique, but on the specific compound, water matrix, and the methodology applied for the analytical measurements. Due to some facts such as the replacement of the instruments and the criteria for the calculation and acceptance of these limits, an evolution of LOQs can also be observed, making the inter-annual comparison more difficult.

Another issue to be faced is presented when trying to assess the risk of produced water in comparison with raw water at the inlet of a DWTP. This analysis can be useful when evaluating the performance of the treatment technologies in removing certain substances. The difficulties arise when some analytes are only measured in one of the water streams as their presence is not expected in the outlet, due to the optimum removal efficiency, or in the inlet, due to their production as result of the treatment of the water flow, e.g. DBPs. The differences in the list of compounds analysed and a change in the limit of quantification, due to the analysis in different water matrixes, pose an additional difficulty in the assessment.

9.5. Conclusions

A methodology was developed in order to globally assess the chemical risk of drinking water and its source water. Indexes were created including those paremetres that have passed all the quality filters (existence of reference toxicological values and concentration measured with a sensitive analytical technique). The average concentration of the paremetres that were excluded from the hazard indexes was compared to the threshold established by legislation.

The annual evolution of the global indexes at the intake and the outlet of a DWTP showed a continuous decrease in the toxicity from 2008 to 2012. After the application of the methodology, resulting global indexes were located below the thresholds except for carcinogenic risk in the output of the DWTP, where the index was slightly above the threshold during 2008 and 2009 before the upgrade of the treatment works with membrane technologies. The annual evolution of indexes shows a decrease in the global values for all situations: H_Q systemic index based on RAIS falls from 0.64 to 0.42 for surface water and from 0.61 to 0.31 for drinking water; R carcinogenic index based on RAIS is negligible for input water and varies between $4.2x10^{-05}$ and $7.4x10^{-06}$ for drinking water; The W systemic index based on WHO moves varies between 0.41 and 0.16 for surface water and between 0.61 and 0.31 for drinking water. A specific analysis for the indexes associated to trihalomethanes (THMs) shows the same pattern.

From the second group of paremetres, not included in the calculation of the indexes, only free chlorine and chlorides at 2008 and 2009, and sodium at 2009 showed average concentrations slightly above the threshold for drinking water.

Although risk indexes were calculated in order to help the decision of the stakeholders in charge of water treatment works and administrations dealing with health issues, it is important not to forget that legislation (e.g. Directive 98/83/EC in Europe) is the main reference when assessing the compliance of water quality to health standards. These indexes have been presented as a tool to show the improvement of produced water, especially after 2009 when the UF and RO membrane technologies were installed.

The methodology developed in the form of risk indexes included more paremetres than those in the legislation in order to provide a tool based on risk assessment and not only on the concentration of legislated paremetres. These indexes take into account different effects (systemic and carcinogenic) and are based on reference values given by international organisations, considering oral ingestion doses. The indexes developed provide a quantification of the quality improvement that could be integrated with Life Cycle Assessment (LCA) and Life Cycles Costing (LCC) analysis.

To summarise, the methodology introduced is able to estimate the risk reduction benefit when a change in the treatment line is introduced and could be used to estimate potential health benefits for this type of investment.

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10

Conclusion

10. Conclusion

The main achievements and impacts related to this thesis are described in this chapter. Progress beyond the state of the art and proposals for future research are also included.

10.1. Scientific achievements

The thesis was developed in different branches of what can be understood as an integrated water quality monitoring strategy. Different methodologies exist for the assessment of the quality of the water and its impact on the health of ecosystems and consumers. The needs of the end users will define which methodology best fits their requirements as each methodology provides information of a different kind and has its own constraints. The best solution may be a combination of methodologies and pursuing different monitoring strategies to meet the same global objective: ensuring public safety and protecting the environment.

The work in this thesis has been executed as part of several projects whose final objectives were focused on improving the water quality monitoring of surface and drinking waters through holistic approaches, that is, by developing methodologies based on different techniques in order to integrate them to achieve the optimum solution. Each of these methodologies has constituted one chapter in this thesis. The following advances are presented as the result of this thesis:

- **Optimisation of methodologies based on off-line techniques in the laboratory including solid phase extraction, liquid chromatography and mass spectrometry for the identification and quantification of a selection of emerging pollutants (pharmaceutical compounds)**

  A selection of pharmaceuticals (28) and oestrogens (10) was made based on previous studies in order to identify their presence along the Llobregat River. Different locations were monitored, estimating the time that the same mass of water would need to arrive to each of the sampling points. This way, the persistence of the compounds and the contribution from subsidiaries and discharges could be taken into account.

  For the detection of emerging compounds at very low levels, a methodology must be based on a very sensitive technique. Currently, only large laboratories have the capacity to perform these

analyses through the use of chromatographic plus mass spectrometry technologies. In this case, the analysis of pharmaceuticals was performed by off-line solid phase extraction (SPE) followed by liquid chromatography tandem mass spectrometry with a triple quadrupole analyser (LC-QqQ-MS/MS). For the analysis of oestrogens, an instrument coupled to the chromatograph in order to automatically perform the extraction and pre-concentration of the compounds from the sample performed SPE.

This technique is very accurate and sensitive, but 2 selected reaction monitoring (SRM) transitions must be recorded for each compound in order to unequivocally confirm its presence. In the case of ibuprofen, gemfibrozil, pravastatin, ketoprofen and ofloxacin, for which only 1 SRM transition was detected due to poor fragmentation of the molecule, additional analysis was proposed through ultra-performance liquid chromatography/mass spectrometry with a time-of-flight analyser (UPLC-TOF-MS). The main constraints of this technique is the lower sensitivity compared to LC-QqQ-MS/MS and the extra cost of performing a second analysis.

Concerning results, 23 out of the 28 pharmaceutical compounds were detected in at least one sample. The highest concentrations were observed for the β-blockers metoprolol and sotalol, the antibiotic ofloxacin and the lipid regulator gemfibrozil. Within the group of oestrogens, only oestrone and oestrone-3-sulphate were positively identified. Oestrone-3-sulphate showed concentrations in some locations considered sufficient to induce oestrogenic effects in aquatic organisms. As a general pattern, the concentration of target compounds increases along the river flow as expected.

- **Integration and validation of emerging biosensing technologies for the on-line automatic measurement of global toxicity of surface waters by inhibition of *Vibrio fischeri* luminescence**

A fully automatic on-line water toxicity monitor called TOXcontrol^{TM}, based on the measurement of the inhibition of luminescence by bacteria, was tested for the first time in the Llobregat River basin. The results for the period studied showed that the toxicity was negligible or even negative mainly due to the low concentration of the toxic substances and the high occurrence of organic matter and nutrients that may act as a catalyst for the metabolism of the bacteria. No special event took place during the testing period to prove the feasibility of the instrument as an Early Warning System.

As very few bibliographic references existed regarding this technology, inhibition curves and EC50 values were calculated for a selection of compounds that can be found in European rivers and drinking waters. EC50 results (in mgL^{-1}) were obtained for nonylphenol (0.03 and 0.06 for 15 and 30 min respectively), triclosan (0.13 and 0.13), terbuthylazine (2.88 and 2.74), dimethoate (6.80 and 6.20), diclofenac (10.26 and 9.82), SDBS (50 and 39), diazinon (193 for 15 min), propanil (1594 for 15 min) and MCPA (2.0 for 15 min). Heavy metals were selected to represent ions that were found in water distribution systems due to leachate from pipe material. In this case, results were performed using copper (II) (10.61 and 4.68), nickel (II) (317 and 222), chromium (III) (190 and 123) and iron (III) (52 for 15 min).

The same organic compounds were tested in an interlaboratory exercise to compare the response of TOXcontrol™ with the results obtained using standardised methodologies based on Microtox™ and *D. magna* tests. The values obtained for *V. fischeri* using the testing instrument were in general in good agreement with those obtained using Microtox™ and those found in the literature. In the case of *D. magna* tests, higher differences were found, particularly for diazinon and nonylphenol. This difference is not unusual, because the technique is based on the response of different organisms.

In the case of metal compounds, a comparison was only carried out between the results obtained using TOXcontrol™ and values reported in the literature using *V. fischeri* techniques. A comparison with the published data identified major differences, providing an indication of the need to standardise the evaluation protocols to achieve reliable data.

The results presented show that the TOXcontrol™ system is accurate and reproducible enough to be used as an on-line automatic alert system. No toxicity response was obtained when testing in real conditions in the Llobregat River waters as the level of toxicants are normally far below EC50 values. This can be seen as an indication of the improvement of the quality of the river during the last decade through the improvement of the wastewater treatments along the basin. Therefore, the system could be used as an early warning system (EWS) able to report abnormal values of toxicity in the river, due to accidental or deliberate contamination, as it is currently used in the Netherlands, Germany, Romania and China (Huangpu River).

In the case of drinking waters, there is an additional constraint. In the case of Barcelona, for example, noticeable levels of chlorine are present in the sample as a result of the disinfection process. The chlorine shows a high toxicity to *V. fischeri* so no alarm could be obtained because of the presence of another toxic substance. However, it is possible to avoid this interference through the use of reagents such as sodium bisulphite so it can be used for such applications.

- **Development of methods based on an in-line UV-Vis spectrophotometer for real-time monitoring of physical-chemical paremetres in river (early warning system) and drinking water (prediction of blends of different sources)**

A UV-Vis probe obtaining a fingerprint of a water sample, that is the spectrum from 200 to 750 nm, was used successfully in two different applications for drinking water quality monitoring.

On one hand, the on-line measurement of surface water could be used as an EWS before the intake of the DWTP if a sudden alteration of water quality occurs. The probe contained previously calibrated models for the transformation of the fingerprint into the following paremetres: turbidity, total organic carbon (TOC), nitrates and SAC-254. The study also attempted to establish correlations between the values obtained from the probe and the analysis performed in the laboratory. This comparison was intended to give information on the performance of the instrument and it could also be used for the local calibration of the probe.

The main difficulty found when validating the probe was the unstable conditions of the Llobregat River. The water matrix changes quickly and the probe was calibrated for a concentration range of paremetres narrower than the range found in real conditions. Moreover, the models used were obtained in surface waters with different characteristics from the Llobregat River. This led to a situation where measures were not recorded during storm episodes (high turbidity but more chance of there being an alarm due to contamination) and correlations not optimum for nitrate and TOC.

The probe was used to measure the water fingerprinting, thereby reflecting the nature of the organic matter. This fingerprint is representative of every water source and was used for the real-time assessment of changes in water quality instead of identifying single contaminants. This information could be used by water managers in charge of distributing drinking water in Barcelona in order to identify the origin of the water in an exact location of the network in real time.

A multivariate model able to describe and distinguish different water sources and blends in the drinking water of Barcelona was obtained from 37 physicochemical paremetres measures in 191 samples from different locations over one year. Another model was built using the fingerprint (absorbance values at 101 different wavelengths) for the same samples. The Principal Component Analysis (PCA) model based on UV-Vis spectral was compared with the PCA model built from

the laboratory physicochemical data. The correlation was good but in order to improve the prediction capability, an analysis of the combination of UV-Vis data with a selection of physicochemical paremetres (conductivity, fluoride and boron) was performed. These paremetres were chosen for the information they provide and for their ability to perform on-line measurements if necessary. Conductivity helps to discriminate between different surface water sources, where boron is an indication of the presence of seawater origin, and fluoride acts a marker for groundwater origin.

Moreover, the influence of the water sources and blending on the occurrence and speciation of different trihalomethanes (THMs) was quantified. Water coming from the Llobregat River, and to a lesser degree from the desalination plant, both with low levels of bromide and iodide, lead to a trend of brominated THMs formation, while water coming from Ter River tends to form chlorinated THMs.

- **Proposal of indexes for measuring the ecological impact of contaminants on aquatic and terrestrial ecosystems**

For the assessment of the potential hazard of the substances found in the Llobregat River waters to the safety of ecosystems, a series of indexes were developed. These indexes allowed us to assess the impact on aquatic organisms and terrestrial vertebrates. The methodology was based on comparing the average concentration of a toxic substance compared to the higher concentration that has no predicted effect on the environment (PNEC).

The list of substances was limited to existing databases for water quality paremetres in the case study location (Catalan Water Administration and Barcelona water utility) and those contaminants where a toxicological reference value could be obtained. Additional data was collected through the analysis of water samples for pesticides and pharmaceutical products in order to include paremetres not analysed routinely but with existing references for their toxic effect in the literature. The PNEC was calculated from the existing toxicological studies divided by an assessment factor (AF). The higher the uncertainty in obtaining the reference value, the higher the AF, so the calculated PNEC would be lower. In this case, the indexes tended to be higher in order to be more conservative when assessing the risk.

Indexes for aquatic organisms were normally higher than the indexes for terrestrial vertebrates indicating that bioconcentration in a single chain level would reduce the impact on vertebrates that are predators of the aquatic organisms. Additionally when regulatory thresholds are used as

reference levels, indexes tended to be lower, as these thresholds are not so conservative because they have been obtained from a significant number of toxicological studies.

According to the results, all the metals studied (barium, copper, nickel and zinc) gave indexes above 1 for aquatic organisms, zinc being the highest, reaching values of 100 and 1000. For terrestrial vertebrates, only zinc showed impact. Concerning organic compounds, the most significant indexes for aquatic organisms referred to the pesticides terbuthylazine, diazinon and MCPA, and the antibiotics clarithromycin and ciprofloxacin. The indexes calculated for terrestrial vertebrates showed no significant impact. When the relation was established using the threshold according to legislation, chlorpyrifos and lindane showed indexes above 1 for some months.

- **Creation of indexes to evaluate the efficiency of water treatment technologies and assess the potential impact of contaminants on drinking water to the supplied population**

Indexes were developed, not only to assess the impact of chemical organic pollutants on the ecosystem, but also to measure the potential hazard of these substances to human health. The methodology used for the assessment considered systemic and carcinogenic effects caused by the oral ingestion of water. A series of concentration values covering up to 261 chemical paremetres over 5 years of raw and treated water in the Sant Joan Despí DWTP was used. The reference data for the calculation of the indexes were obtained from the databases developed by the World Health Organisation (WHO) and the Risk Assessment Information System (RAIS).

A tool was created using the Microsoft Excel™ programme for the calculation of risk indexes. Once the data on monthly average concentrations were loaded, the tool automatically presented the global risk for each year through the addition of individual risk indexes after performing some filtering. For the calculation of annual concentration averages, the tool attributed a value of half the limit of quantification (LOQ/2) to those concentrations below LOQ. Filtering was applied in order to exclude those contaminants with no toxicity reference data and those whose concentration had been obtained from analytical techniques that were not sensitive enough. This last filter avoided an overestimation of the global risk when individual risks were obtained from high LOQ/2 values.

The annual evolution of global indexes showed a decrease in the global values for all situations in the 5-year period: the systemic index based on RAIS fell from 0.64 to 0.42 for surface water and from 0.61 to 0.31 for drinking water; carcinogenic index based on RAIS was negligible for input water and varied between $4.2x10^{-05}$ and $7.4x10^{-06}$ for drinking water; the systemic index based on

WHO varied between 0.41 and 0.16 for surface water and between 0.61 and 0.31 for drinking water. All the resulting global indexes were located below the thresholds except for the carcinogenic risk at the output during 2008 and 2009 when the index was slightly above the threshold. A specific analysis for the indexes associated to THMs showed the same pattern.

These indexes showed the improvement of the produced water, especially after 2009 when the ultrafiltration (UF) and reverse osmosis (RO) membrane technologies were installed in the DWTP.

For systemic risk, the compounds with a higher contribution to the indexes at the inlet were nitrates and nitrites. Bromates, strontium, boron, barium and nickel also contributed considerably. At the outlet, boron and the halogenated chlorodibromomethane, bromoform, trichloroethene and 1,1,2-trichlorethane appeared at the highest position along with the former compounds. For carcinogenic risk, no significant risk was shown at the inlet. The risk was higher at the outlet, as DBPs can only be found in treated water. Legislated THMs and 1,1,2-Trichloroethane made the maximum contribution to the general risk.

The paremetres excluded at the filtering process were compared to the drinking water legislation. Only free chlorine and chlorides in 2008 and 2009, and sodium in 2009 showed average concentrations slightly above the thresholds.

10.2. Possible future research

New methodologies are constantly being developed to analyse new substances in the laboratory that are suspected to be potentially harmful. Recent publications report the increasing use of nanoparticles used in industry and the growing concern about their impact on the environment. For example, the occurrence of fullerenes has been reported recently in the vicinities of Barcelona (Sanchís et al., 2014). Legislation such as the European Directive for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) contributes to a need to assess and manage the risks posed by chemicals and the need to properly monitor them.

Although laboratory analytical methodologies tend to be more sensitive than sensors working on line, these new emerging substances are present in concentration orders of even pgL^{-1}. Pre-concentration techniques, such as SPE, help to reduce the detection limits of laboratory instruments.

Other techniques exist in order to perform this concentration on site. Passive sampling techniques, which simulate bioaccumulation organisms, are composed of a sorbent material that collects pollutants in proportion to time and concentration. They have shown themselves to be a reliable tool to allow the detection of levels much lower than those obtained by the traditional analysis of spot water samples (Allan et al., 2006). New technology has been developed in this field by the use of an integrative sampling device that consists of a small peristaltic pump controlled by an electronic board that governs its operation by pre-programming. A constant flow passes through a glass cell containing adsorbent material, thereby overcoming the problems related to turbulences in the water flow (Santiago Sánchez et al., 2014).

The analysis of pharmaceutical compounds and oestrogens in this thesis confirms the presence of some of the target compounds in concentrations that could lead to a potential risk to the environment. Further studies on the risk of these and other substances need to be undertaken. As has been previously stated in this thesis, the development of analytical techniques should be accompanied by an increasing number of toxicological studies so that more accurate risk assessments can be performed.

On-line detection systems, such as the commercial devices tested in this thesis, are generally designed to provide fast but not accurate data on the quality of water. A network of instruments needs to be installed in the most sensitive locations of a system, these being a water basin or a drinking water production and distribution network. Ideally, these systems should attempt to characterise the contamination event by identifying the contaminant or its class, indicating the concentration of the contaminant, calculating its spread within the system, and determining the duration of the event (ASCE, 2004).

The main problems that were found when testing the on-line instruments could be grouped into two areas:

- Lack of sensitivity. The instruments were designed to perform the measurement of the target paremetre (or surrogate) in a small amount of a water sample. Due to the fact that some contaminants are present in very low concentrations in surface waters, and even lower in drinking waters, the required sensitivity is not achieved in a significant number of cases. Increasing the volume of the sample could improve the LOQ but would also lead to some problems related to the presence of interfering substances or the matrix itself and, in some instruments, a higher consumption of reagents.

- Lack of adaption to local conditions. The instruments were normally designed and validated to work on a specific environment. The longer the time the device is on the market, the more experience is gained in different situations and specific adaptations may be designed. These adaptations may be in the hardware or in the software used to convert the signal into a required paremetre.

In the case of TOXcontrol™, a pre-concentration system needs to be adapted to analyse larger volumes of water. A prototype of an on-line SPE instrument was tested as part of this thesis but the results showed low recoveries and low reproducibility. Moreover, the time for the analysis rose from 45 minutes to 2 or 3 hours. In order to solve this problem, on-going projects have been focused on developing and validating a new SPE system.

Additionally, new tests could be done in order to discover the response of the instrument to new compounds. The toxicity of both sulphapyridine and its acetylated metabolite was evaluated for the first time by using TOXcontrol™ as part of a collaborative research project. Inhibition curves were obtained and a EC50 of 27.4 mgL-1 was observed for sulphapyridine and 8.2 mgL-1 for its metabolite. The results were published by (García-Galán et al., 2012). As more toxicological studies are performed based on *V. fischeri*, the more feasible it will be to establish comparisons with other results and to assess the accuracy of the instrument.

In the case of spectro::lyser™, as it is an in-line probe that works submerged in the water flow, no pre-concentration system could be attached, unless the probe was operated in an on-line mode by the use of a flow cell. In this case, working with different optical lengths could help to partially solve the problem of sensitivity. A large optical length should be used in situations with a very clear matrix e.g. drinking water, while a shorter length might be used when dealing with surface water.

In our case, the Llobregat River, the ideal situation would be to work with two probes with different optical length. At situations with no rainfall, that is, low turbidity, a wider optical length would make it possible to detect compounds with higher sensitivity. During storm events, when turbidity is much higher, a narrower optical length would mean a loss of sensitivity but would make it possible to record data even though turbidity increases.

Another strategy needed in order to adapt to local conditions is the development of prediction models based on local data. Global calibration models can be adjusted to local data but if these models have been created in very different environments it is advisable to create new ones. A new model for the prediction of nitrates was created for the Llobregat River (results not published). The results showed a more accurate prediction model than the one supplied using the probe. The main problem was related to the fact that the new model was external, so no automatic real-time information on nitrates concentration could be obtained, as fingerprints needed to be downloaded to work with them. Discussions have been initiated with the instrument developers in order to assess the feasibility of uploading self-developed prediction models.

According to Bogue (2008), academic and industrial research groups are working on the development of new sensors motivated by the desire to replace some laboratory determinations with on-site measurements; the need for more sensitive sensors in response to falling limits; and the requirement for more data to provide spatial coverage. Some considerations such as the initial investment and the operational cost should be compared against the benefits arising from the ability to obtain data more rapidly.

As the optimal on-line instrument does not currently exist, the approaches that are most likely to succeed will involve one tier of instruments to detect contamination events and provide location information, while a second tier (which will probably require the laboratory analysis of samples) will be needed to identify and measure the specific contaminants (ASCE, 2004).

PNEC calculations are based on the numbers of toxicological studies and the information they provide (e.g. long term effects instead of only acute toxicity). More information on toxicological effects will lead to more accurate PNEC calculations. Toxicological information not currently available on new compounds plus the acquisition of concentration values of new substances due to an improvement of the sensitivity of analytical techniques will lead to the creation of new indexes. As the toxicological information is more accurate, more compounds will be able to be included in the list of priority pollutants. This will mean the creation of new EQS that are less conservative than predicted PNEC. The inclusion of a substance as a priority pollutant may decrease its ecological impact, depending on the calculations performed.

Progress on the risk assessment regarding the effect of pollutants in ecosystems may lead to a situation where individual risks may be lowered due to the reduction of the uncertainty in PNEC calculations, but at the same time, the global risk may appear to be increased due to the inclusion of new substances.

In the case of the indexes developed in this thesis to measure the impact of water pollutants on human health, the global values may rise in the future due to the inclusion of compounds that were rejected in the filtering steps. If new reference data are included in the RAIS and WHO databases and the LOQ of the analytical techniques are improved, more individual risks values would contribute to the global one. Although the number of included paremetres grows, the thresholds for the global risks values will remain the same (the ones established for individual risks, a ratio of 1 for systemic risk and a ratio of 10^{-5} for carcinogenic risk). This fact may lead to a situation where added values will rise above the thresholds even if the situation improves. A new threshold could be established when aggregating indexes, e.g. a value considering the number of paremetres included. Otherwise, this will lead to a situation where sites with more sophisticated and complete monitoring programmes will be disadvantaged.

10.3. Expected impacts

10.3.1. Impact of the state of the art of the technology

The research included in this thesis was performed using the best technology available at the time when the projects were executed. A benchmark was established for every project in order to select the technology that best suited the needs of the end users from between the most advanced technologies.

The performance of tests at the end users' facilities according to their needs helps to spread these technologies and their uptake in the market. For this reason, it is important to publish the results although it is not a common practice when the end users themselves perform the tests. Recommendations included in the publications will help the developers to improve their instruments or to design new ones.

In the analysis of emerging compounds, the most accurate and sensitive technologies available in the laboratory were selected. It was important to be able to identify a whole family of compounds using a unique analysis in order to optimise cost and time. A review of the literature showed that HPLC-QqQ-MS/MS was the most adequate technique due to its capacity to obtain two product ions from the parent one; therefore 2 transitions could be observed, making the technique suitable for providing the level of confirmation needed. When 2 transitions cannot be obtained, due to the poor fragmentation of the ion, an additional technique may be used in order to perform an unequivocal confirmation. In this case, UPLC-TOF-MS was used due to the high accuracy of this technique although sensitivity was not as high as the previous one. Apart from triple quadrupole (QqQ) and time-of-flight (TOF), other similar mass spectrometry techniques have been used recently to analyse similar compounds, such as the ion trap and LTQ-Orbitrap XL mass spectrometer (Haddad and Kümmerer, 2014).

Concerning on-line selected instruments; the use of optical devices is increasing. The main advantages of these technologies are related to their low maintenance and, in the case of spectrophotometry, the use of reagent free techniques. Multi and hyperspectral technologies are considered multiparemetre sensors and several paremetres can be obtained by developing the proper calibration models. The possibilities of working with fingerprints through the use of chemometrics will make it possible to extract the information that might be useful for the end user. Any substance in water that has a response in the range the device is measuring (IR, Visible or UV) can be monitored. Alternatively, there are strategies that base the monitoring on the shape of the fingerprint without the intention of obtaining indirect paremetres. Other instruments such as TOXcontrolTM and ColiguardTM, although using reagents to obtain a response, perform their detection through optical techniques.

10.3.2. Impact of the case study

Thanks to the projects developed, monitoring platforms were tested in Barcelona for surface and drinking waters. End users could benefit from the results because:

- Selected instruments were tested and adapted to local conditions, plus specific calibration models were developed
- Knowledge was gained on water quality paremetres and their effects on the health of ecosystems and the public

Results from the monitoring performed confirmed the presence of high consumption drugs. Significant levels were found for the ß-blockers metoprolol and sotalol, the antibiotic ofloxacin, the lipid regulator gemfibrozil, and the oestrogens oestrone and oestrone-3-sulphate. Oestrone levels were in some sites close to those considered sufficient to cause oestrogenic effects in aquatic organisms. However, the most polluted waters are currently diverted and discharged to the river at a location downstream of the intake of the Sant Joan Despí DWTP.

In the case of drinking water distribution, the results of the work confirmed that a tool based on the real-time measurement of some paremetres and their subsequent chemometric analysis could be of great help in the operation of complex drinking water distribution systems.

Concerning the methodology introduced based on the assessment of the hazard of water pollutants to the consumers; an estimation of the risk reduction benefit when a change to the treatment line is introduced was achieved. The methodology could be used to estimate potential health benefits for any investment. The indexes developed provide a quantification of the quality improvement that could be integrated with Life Cycle Assessment (LCA) and Life Cycles Costing (LCC) analysis. This study could help to develop new managing practices based, not only on the occurrence itself, but also on the potential hazard of the chemical contaminants.

10.3.3. Impact of legislation

Legislation on natural and drinking waters is evolving constantly. Paremetres to be measured in surface waters have recently increased from 33 to 45 (2013/39/EU). Apart from this list of priority pollutants, a watch list has been defined. Currently, this list includes 3 pharmaceutical compounds (estradiol,

ethynylestradiol and diclofenac) but more compounds will be included soon (a draft of this extended watch list currently exists). Methodologies for analysing these paremetres need to be ready.

Moreover, some guidelines recommend a monitoring strategy based on risk assessment and the measurement of global paremetres in order to decrease the cost of an increasing number of substances and for monitoring to be more locally adapted. The thesis provides public administrations and water operators with the tools that could be the basis of new managing strategies for decision-making.

10.4. References

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